

### Priority of the Invention

## Background of the Invention

25 Research over the past several years has revealed that asthma likely results from complex interactions among inflammatory cells, mediators, and other cells and tissues resident in the airway. Mast cells, eosinophils, epithelial cells, macrophage, and activated T-cells all play an important role in the inflammatory process associated with asthma (Djukanovic et al., *Am. Rev. Respir. Dis.*; 142:434-457; 1990). It is believed that these cells  
30 can influence airway function through secretion of preformed and newly synthesized mediators which can act directly or indirectly on the local tissue. It has also been recognized that subpopulations of T-lymphocytes (TH-2) play an important role in regulating allergic inflammation in the airway by releasing selective cytokines and establishing disease chronicity (Robinson, et al. *N. Engl. J. Med.*; 326:298-304; 1992).

Asthma is a complex disorder which arises at different stages in development and can be classified based on the degree of symptoms of acute, subacute or chronic. An acute inflammatory response is associated with an early recruitment of cells into the airway. The subacute inflammatory response involves the recruitment of cells as well as the activation of resident cells causing a more persistent pattern of inflammation. Chronic inflammatory response is characterized by a persistent level of cell damage and an ongoing repair process, which may result in permanent abnormalities in the airway.

Medications for the treatment of asthma are generally separated into two categories, quick-relief medications and long-term control medications. Asthma patients take the long-term control medications on a daily basis to achieve and maintain control of persistent asthma. Long-term control medications include anti-inflammatory agents such as corticosteroids, chromolyn sodium and medacromil; long-acting bronchodilators, such as long-acting  $\beta_2$ -agonists and methylxanthines; and leukotriene modifiers. The quick-relief medications include short-acting  $\beta_2$  agonists, anti-cholinergics, and systemic corticosteroids. There are many side effects associated with each of these drugs and none of the drugs alone or in combination is capable of preventing or completely treating asthma.

Allergy is a disease associated with the production of antibodies from a particular class of immunoglobulin, IgE, against allergens. The development of an IgE-mediated response to common aeroallergens is also a factor which indicates predisposition towards the development of asthma. If an allergen encounters a specific IgE which is bound to an Fc IgE receptor on the surface of a basophil (circulating in the blood) or mast cell (dispersed throughout solid tissue), the cell becomes activated, resulting in the production and release of mediators such as histamine, serotonin, and lipid mediators. Allergic diseases include but are not limited to rhinitis (hay fever) asthma, urticaria and atopic dermatitis.

Conventional methods for treating or preventing allergy have involved the use of anti-histamines or desensitization therapies. Anti-histamines and other drugs which block the effects of chemical mediators of the allergic reaction help to regulate the severity of the allergic symptoms but do not prevent the allergic reaction and have no effect on subsequent allergic responses. Desensitization therapies are performed by giving small doses of an allergen, usually by injection under the skin, in order to induce an IgG-type response against the allergen. The presence of IgG antibody helps to neutralize the production of mediators resulting from the induction of IgE antibodies, it is believed. Initially, the subject is treated with a very low dose of the allergen to avoid inducing a severe reaction and the dose is slowly

increased. This type of therapy is dangerous because the subject is actually administered the compounds which cause the allergic response and severe allergic reactions can result.

### **Summary of the Invention**

Improved methods and products for the prevention and/or treatment of asthma and allergy are provided according to the invention. The invention is based, in some aspects, on the finding that when immunostimulatory nucleic acid molecules are used in conjunction with medicaments for the treatment of asthma and allergy, some unexpected and improved results are observed. For instance, the efficacy of the combination of immunostimulatory nucleic acids and asthma and allergy medicaments is profoundly improved over the use of each of the medicaments alone. The results are surprising in part because the drugs act through different mechanisms and would not necessarily be expected to improve the efficacy of one another in a synergistic manner.

In some aspects, the invention is a method for preventing or treating asthma or allergy by administering a synergistic combination of an immunostimulatory nucleic acid and an asthma/allergy medicament, wherein the combination is administered in an effective amount for synergistically reducing the immune or inflammatory response caused by a mediator of asthma or allergy. It was surprisingly discovered according to the invention that the combination of the immunostimulatory nucleic acid and the asthma/allergy medicament worked synergistically to reduce the immune or inflammatory response initiated when a mediator of asthma or allergy is encountered.

In other aspects, the invention is a method for altering the dosage of the asthma/allergy medicament that is required to treat a subject suffering from asthma or allergy. The invention in one aspect is a method for increasing the dose of an asthma/allergy medicament without inducing the level of side effects ordinarily observed with that dose of an asthma/allergy medicament. The method is accomplished by administering to a subject suffering from asthma or allergy or at risk of developing asthma or allergy, an asthma/allergy medicament in a dose which would ordinarily induce side effects, administering an immunostimulatory nucleic acid to the subject, wherein administration of the immunostimulatory nucleic acid prevents the side effects associated with the high dose of the asthma/allergy medicament. The method provides a basis for administering higher therapeutic doses of an asthma/allergy medicament to a subject in order to prevent or reduce the symptoms associated with an asthmatic or an allergic response more sufficiently than a lower dose. It is not desirable to

administer such high doses alone, in the absence of the immunostimulatory nucleic acid, because of the side effects resulting from the high dose.

In another aspect, the invention includes a method for decreasing the dose of an asthma/allergy medicament by administering to a subject having asthma or allergy or at risk of developing asthma or allergy an asthma/allergy medicament in a sub-therapeutic dosage and an immunostimulatory nucleic acid, wherein the combination of the sub-therapeutic dose of the asthma/allergy medicament and the immunostimulatory nucleic acid produce a therapeutic result in the prevention or treatment of asthma or allergy in the subject. The method allows a lower dose of the asthma/allergy medicament to be used. This provides several advantages, including lower costs associated with using less drugs and less chances of inducing side effects resulting from the medications by using lower doses.

According to other aspects, the invention involves methods for treating or preventing asthma and/or allergy by administering an immunostimulatory nucleic acid and an asthma/allergy medicament in different dosing schedules. In one aspect, the invention is a method for preventing or treating asthma or allergy by administering to a subject an effective amount of an immunostimulatory nucleic acid in an effective amount for producing the immune response and subsequently administering to the subject an asthma/allergy medicament. In other aspects, the invention is a method for preventing or treating asthma or allergy by administering to a subject an allergy/asthma medicament in an effective amount for providing some symptomatic relief and subsequently administering an immunostimulatory nucleic acid to the subject. In some embodiments, the immunostimulatory nucleic acid is administered in an effective amount for redirecting the immune response from a Th2 to a Th1 immune response. In some embodiments, the immunostimulatory nucleic acid is administered consistently over a period of time, such as, for instance, in a sustained release vehicle.

In another aspect of the invention is a method for treating asthma or allergy by administering to a subject having asthma or allergy or at risk of developing asthma or allergy an immunostimulatory nucleic acid and an asthma/allergy medicament, wherein the immunostimulatory nucleic acid is administered systemically and the asthma/allergy medicament is administered locally. In yet another aspect, the immunostimulatory nucleic acid is administered locally and the asthma/allergy medicament is administered systemically.

According to yet another aspect of the invention, a method for treating or preventing asthma/allergy is provided. The method is accomplished by administering to a subject having

asthma or allergy or at risk of developing asthma or allergy, an immunostimulatory nucleic acid and an asthma/allergy medicament on a routine schedule. In some embodiments, the routine schedule is a daily, weekly, monthly, or quarterly administration of the medicaments. In other embodiments, the immunostimulatory nucleic acid and/or the asthma/allergy medicament is administered in two or more doses.

The immunostimulatory nucleic acid can be administered on a recurring basis, such as daily, weekly, or monthly in one or more doses. Alternatively, it can be administered on a non-regular basis e.g. whenever symptoms being. In yet other embodiments, the asthma/allergy medicament is a quick relief asthma/allergy medicament and in other embodiments it is a long-lasting asthma/allergy medicament.

According to yet another aspect of the invention, methods for treating or preventing asthma or allergy using specific immunostimulatory nucleic acid molecules are provided. The method in one aspect involves a method for preventing or treating asthma or allergy by administering to a subject suffering from asthma or allergy or at risk of developing asthma or allergy, an immunostimulatory nucleic acid having a sequence selected from the group consisting of SEQ ID NO:1 through to SEQ ID NO:1093 and administering to the subject an asthma/allergy medicament.

In yet another aspect of the invention, a method for preventing or treating asthma or allergy utilizing different routes of administration is provided. In one aspect, the method involves the step of administering to a subject having asthma or allergy or at risk of developing asthma or allergy, an immunostimulatory nucleic acid, wherein the immunostimulatory is administered systemically and wherein the asthma/allergy medicament is administered locally. In a related embodiment, the immunostimulatory nucleic acid molecule may be administered locally and the asthma/allergy medicament is administered systemically. In still other embodiments, the immunostimulatory nucleic acid and the asthma/allergy medicament are administered by the same route (i.e., both delivered locally or both delivered systemically), and optionally at the same time.

The invention according to another aspect is a method of preventing or treating asthma or allergy by administering a poly-G nucleic acid, in an effective amount for treating or preventing asthma or allergy. In some embodiments the poly-G nucleic acid is administered alone and in other embodiments the poly-G nucleic acid is administered in conjunction with an asthma/allergy medicament. The poly-G nucleic acid in preferred embodiments comprises one of the following formulas: 5' X<sub>1</sub>X<sub>2</sub>GGGX<sub>3</sub>X<sub>4</sub>3', wherein X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, and X<sub>4</sub> are

nucleotides, 5' GGGNNGG 3' or 5' GGGNNGGNGGG 3', wherein N represents between 0 and 20 nucleotides. In some embodiments at least one of X<sub>3</sub> and X<sub>4</sub> are a G and in other embodiments both of X<sub>3</sub> and X<sub>4</sub> are a G. Accordingly, in some embodiments, the poly-G nucleic acid may comprise a sequence of 5' X<sub>1</sub>X<sub>2</sub>GGGGX<sub>4</sub> 3'. In still other embodiments, the poly-G nucleic acid is one which is rich in G (e.g., six out of seven bases are G, or six out of eight bases are G).

The poly-G may be free of unmethylated CG dinucleotides, or may include at least one unmethylated CG dinucleotide.

The poly G nucleic acid in some embodiments is selected from the group consisting of SEQ ID NO: 5, 6, 73, 215, 267-269, 276, 282, 288, 297-299, 355, 359, 386, 387, 444, 476, 531, 557-559, 733, 768, 795, 796, 914-925, 928-931, 933-936, and 938. In other embodiments the poly G nucleic acid includes a sequence selected from the group consisting of SEQ ID NO: 67, 80-82, 141, 147, 148, 173, 178, 183, 185, 214, 224, 264, 265, 315, 329, 434, 435, 475, 519, 521-524, 526, 527, 535, 554, 565, 609, 628, 660, 661, 662, 725, 767, 825, 856, 857, 876, 892, 909, 926, 927, 932, and 937.

The invention provides, in yet another aspect, a method for treating or preventing asthma or allergy in a hypo-responsive subject. The method involves administering to a hypo-responsive subject having asthma or allergy or at risk of developing asthma or allergy an immunostimulatory nucleic acid. In one embodiment, the method further comprises administering to the hypo-responsive subject an asthma/allergy medicament. If the asthma/allergy medicament is not administered to the hypo-responsive subject, then the immunostimulatory nucleic acid is administered in an amount to treat or prevent the asthma or allergy. If the asthma/allergy medicament is administered to the hypo-responsive subject, then the immunostimulatory nucleic acid and the asthma/allergy medicament are administered in an effective amount to treat or prevent the asthma or allergy. In this latter instance, the amount of the immunostimulatory nucleic acid and the amount of the asthma/allergy medicament may be insufficient (i.e., ineffective) in treating or preventing the asthma or allergy if administered alone. In other words, in some embodiments, the immunostimulatory nucleic acid may be administered to the hypo-responsive subject in a sub-therapeutic amount. Similarly, the asthma/allergy medicament may also be administered in a sub-therapeutic amount. However, the combination of the immunostimulatory nucleic acid and the asthma/allergy medicament allows for lower doses of one or both in order to treat or prevent

the asthma or allergy. The immunostimulatory nucleic acid may be administered concurrently with the asthma/allergy medicament, but need not be.

The hypo-responsive subject may be one who is hypo-responsive to an asthma/allergy medicament. In one embodiment, the hypo-responsive subject is selected from the group  
5 consisting of a subject who is refractory to an asthma/allergy medicament, a subject who is a non-responder to an asthma/allergy medicament, an elderly subject and a neonatal subject.

According to yet another aspect of the invention, a method is provided for preventing asthma or allergy in a subject at risk of developing asthma or allergy which involves administering to a subject at risk of developing asthma or allergy an effective amount of an  
10 immunostimulatory nucleic acid substantially prior to an asthmatic or an allergic event.

In one embodiment, the immunostimulatory nucleic acid is administered at least three months, at least two months, at least one month, or at least 20 days prior to the asthmatic or allergic event. In another embodiment, the immunostimulatory nucleic acid is administered at least two weeks prior to the asthmatic or allergic event. In yet another embodiment, the  
15 immunostimulatory nucleic acid is administered at least 10 days, at least 5 days or at least 2 days prior to the asthmatic or allergic event.

In one embodiment, the asthmatic or allergic event is selected from the group consisting of an asthma attack, seasonal allergic rhinitis, and perennial allergic rhinitis.

In one embodiment, the immunostimulatory nucleic acid is administered in a routine schedule. In a related embodiment, the routine schedule is selected from the group consisting  
20 of a daily routine, a weekly routine, a bi-weekly routine, a monthly routine, and a bi-monthly routine.

In a further aspect, the invention provides another method for decreasing a dose of an asthma/allergy medicament. The method involves administering to a subject at risk of  
25 developing asthma or allergy, substantially prior to an asthmatic or allergic event, an immunostimulatory nucleic acid in an amount to decrease an effective amount of an asthma/allergy medicament subsequently administered to the subject in order to treat the asthma or allergy.

In one embodiment, the immunostimulatory nucleic acid is administered at least three  
30 months, at least two months, at least one month or at least 20 days prior to the asthmatic or allergic event. In another embodiment, the immunostimulatory nucleic acid is administered at least two weeks, at least 10 days, at least one week, at least 5 days or at least 2 days prior to the asthmatic or allergic event.

In one embodiment, the asthmatic or allergic event is selected from the group consisting of an asthma attack, seasonal allergic rhinitis, and perennial allergic rhinitis.

In one embodiment, the immunostimulatory nucleic acid is administered in a routine schedule. The routine schedule may be selected from the group consisting of a daily routine, a weekly routine, a bi-weekly routine, a monthly routine, and a bi-monthly routine.

The method may further comprise administering to the subject the asthma/allergy medicament subsequent to the administration of the immunostimulatory nucleic acid. In one embodiment, the asthma/allergy medicament is administered immediately prior to, concurrently with, or following the asthmatic or allergic event. The method may further comprise administering the immunostimulatory nucleic acid concurrently with or following the asthmatic or allergic event. In one embodiment, the immunostimulatory nucleic acid is administered concurrently with the asthma/allergy medicament. In one embodiment, the asthma/allergy medicament is administered in a sub-therapeutic dose.

In these and other aspects of the invention, the immunostimulatory nucleic acids have a number of attributes. The immunostimulatory nucleic acids may have a modified backbone. In some embodiments, the modified backbone is a phosphate modified backbone, and in related embodiments, the phosphate modified backbone is a phosphorothioate backbone. In certain embodiments, the immunostimulatory nucleic acid is a CpG nucleic acid, in other embodiments, the immunostimulatory nucleic acid is a T-rich nucleic acid, while in still other embodiments, the immunostimulatory nucleic acid is a poly-G nucleic acid. Preferably, the T-rich and poly-G nucleic acids are also CpG nucleic acids. In still other embodiments, the immunostimulatory nucleic acid comprises a poly-G motif (e.g., 5' GGGG 3') and a palindrome. Preferably, the immunostimulatory nucleic acid comprises two poly-G motifs, one 5' and one 3' to a centrally located palindrome sequence. Even more preferably, the backbone of these latter immunostimulatory nucleic acids is chimeric (i.e., it is partially, but not completely, composed of phosphorothioate linkages). In some embodiments, a plurality of immunostimulatory nucleic acids is administered, wherein the plurality comprises CpG nucleic acids and T-rich nucleic acids, or CpG nucleic acids and poly-G nucleic acids, or T-rich nucleic acids and poly-G nucleic acids.

In these and other aspects of the invention, the asthma/allergy medicaments have a number of attributes. In some embodiments, the asthma/allergy medicament is an asthma medicament, while in still other embodiments, the asthma/allergy medicament is an allergy medicament.



In some embodiments, the asthma/allergy medicament is selected from the group consisting of a steroid and an immunomodulator. In certain embodiments, the steroid may be selected from the group consisting of beclomethasone, fluticasone, tramcinolone, budesonide, and budesonide. In certain embodiments, the immunomodulator may be selected from the group consisting of an anti-inflammatory agent, a leukotriene antagonist, an IL-4 mutein, a soluble IL-4 receptor, an immunosuppressant, anti-IL-4 antibody, an IL-4 antagonist, an anti-IL-5 antibody, a soluble IL-13 receptor-Fc fusion protein, an anti-IL-9 antibody, a CCR3 antagonist, a CCR5 antagonist, a VLA-4 inhibitor, and a downregulator of IgE. The downregulator of IgE may be an anti-Ig antibody or a fragment thereof, but need not be so limited. The immunosuppressant may be a tolerizing peptide vaccine, but need not be so limited.

In some embodiments, the asthma/allergy medicament is a medicament selected from the group consisting of a PDE-4 inhibitor, a bronchodilator/beta-2 agonist, a K<sup>+</sup> channel opener, a VLA-4 antagonist, a neurokin antagonist, a TXA<sub>2</sub> synthesis inhibitor, Xanthanine, an arachidonic acid antagonist, a 5 lipoxygenase inhibitor, a thromboxin A<sub>2</sub> receptor antagonist, a thromboxane A<sub>2</sub> antagonist, an inhibitor of 5-lipoxygenase activation protein, and a protease inhibitor. In certain embodiments, the bronchodilator/beta-2 agonist may be selected from the group consisting of salmeterol, salbutamol, terbutaline, D2522/formoterol, fenoterol and orciprenaline.

In some embodiments, the asthma/allergy medicament is a medicament selected from the group consisting of an anti-histamine and a prostaglandin inducer. In certain embodiments, the anti-histamine is selected from the group consisting of loratidine, cetirizine, buclizine, ceterizine analogues, fexofenadine, terfenadine, desloratadine, norastemizole, epinastine, ebastine, ebastine, astemizole, levocabastine, azelastine, tranilast, terfenadine, mizolastine, betastastine, CS 560 and HSR 609. The prostaglandin inducer may be S-5751, but is not so limited.

In still other embodiments, the asthma/allergy medicament is a prostaglandin inhibitor in the form of a cyclooxygenase-2 (COX-2) inhibitor. The COX-2 inhibitor may be selected from the group consisting of celecoxib, rofecoxib, NS-398, l-745,337, meloxicam, nimesulide, SC236, and C-phycocyanin.

A composition comprising a poly-G nucleic acid in an aerosol formulation is provided according to other aspects of the invention.

A kit is provided according to another aspect of the invention. The kit in one aspect includes a sustained-release vehicle containing an immunostimulatory nucleic acid and at least one container housing an asthma/allergy medicament, and instructions for timing of administration of the immunostimulatory nucleic acid and the asthma/allergy medicament. In  
5 another aspect, the kit includes containers for multiple administrations of immunostimulatory nucleic acid and/or multiple administrations of immunostimulatory nucleic acid and at least one container housing an asthma/allergy medicament.

A composition is provided according to another aspect of the invention. The composition includes an immunostimulatory nucleic acid and an asthma/allergy medicament,  
10 formulated in a pharmaceutically-acceptable carrier and in an effective amount for preventing or treating an immune response associated with exposure to a mediator of asthma or allergy.

Formulations of poly-G nucleic acids are also encompassed by the invention. For instance the invention includes a pharmaceutical composition of a poly-G nucleic acid in an aerosol formulation.

15 The immunostimulatory nucleic acid may be any of the immunostimulatory nucleic acids described above, and may have any of the attributes of the immunostimulatory nucleic acids described above which are useful in other aspects of the invention.

The asthma/allergy medicament may be any of the asthma medicaments or allergy medicaments described above which are useful in other aspects of the invention.

20 Each of the limitations of the invention can encompass various embodiments of the invention. It is, therefore, anticipated that each of the limitations of the invention involving any one element or combinations of elements can be included in each aspect of the invention.

### **Detailed Description of the Invention**

The invention relates to methods and products for the treatment of asthma/allergy  
25 using a combination of immunostimulatory nucleic acids and asthma/allergy medicaments. The compositions can be administered in higher doses without as many side effects as are ordinarily achieved at those dosage levels or in lower doses with higher efficacy than is ordinarily achieved with those doses. The compositions can also be administered on fixed schedules or in different temporal relationships to one another. The various combinations  
30 have many advantages over the prior art methods of treating asthma and allergy.

One method for treating or preventing asthma or allergy includes the step of administering a synergistic combination of an immunostimulatory nucleic acid and an asthma/allergy medicament in an effective amount to treat or prevent the asthma or allergy.

An "immunostimulatory nucleic acid" as used herein is any nucleic acid containing an immunostimulatory motif or backbone that induces a Th1 immune response and/or suppresses a Th2 immune response. Immunostimulatory motifs include, but are not limited to, CpG motifs, poly-G motifs, and T-rich motifs. Immunostimulatory backbones include, but are not limited to, phosphate modified backbones, such as phosphorothioate backbones. Immunostimulatory nucleic acids have been described extensively in the prior art and a brief summary of these nucleic acids is presented below.

The immunostimulatory nucleic acids when combined with the asthma/allergy medicaments have many advantages over each composition alone for the treatment of asthma and allergy. The immunostimulatory nucleic acid functions in some aspects by simultaneously suppressing Th2-type immune responses (IL-4, IgE production, histamine release) that can result in airway inflammation and bronchial spasm, and/or inducing Th1-type immune responses (IFN- $\gamma$  and IL-12 production) that promote harmless antibody and cellular responses. This creates an environment inside the body that safely and effectively prevents hypersensitive reactions from occurring, thereby eliminating symptoms.

The immunostimulatory nucleic acids eliminate/reduce bronchial hyperreactivity, bronchoconstriction, bronchial obstruction, airway inflammation and atopy (which improves asthma control, normalizes lung function, prevents irreversible airway injury); and may also inhibit acute response to exercise, cold dry air, and SO<sub>2</sub>. The nucleic acids provide long-lasting effects, thus reducing dosing regimes, improving compliance and maintenance therapy, reducing emergency situations; and improving quality of life. These compounds are also useful because they provide early anti-infective activity, which leads to decreasing infectious episodes, which further reduces hyperreactive immune responses. This is especially true in subjects like children or immuno-compromised subjects. Furthermore, use of the immunostimulatory nucleic acids reduces/eliminates use of inhalers, which can exacerbate hypersensitive reactions by providing simpler and safer delivery and by allowing less drugs to be used.

Immunostimulatory nucleic acids stimulate the immune system to prevent or treat allergy and/or asthma. The strong yet balanced, cellular and humoral immune responses that result from the nucleic acid's stimulation reflect the body's own natural defense system against invading allergens and initiators.

The terms "nucleic acid" and "oligonucleotide" are used interchangeably to mean multiple nucleotides (i.e. molecules comprising a sugar (e.g. ribose or deoxyribose) linked to

a phosphate group and to an exchangeable organic base, which is either a substituted pyrimidine (e.g. cytosine (C), thymine (T) or uracil (U)) or a substituted purine (e.g. adenine (A) or guanine (G)). As used herein, the terms refer to oligoribonucleotides as well as oligodeoxyribonucleotides. The terms shall also include polynucleosides (i.e. a polynucleotide minus the phosphate) and any other organic base containing polymer. Nucleic acids include vectors, e.g., plasmids as well as oligonucleotides. Nucleic acid molecules can be obtained from existing nucleic acid sources (e.g. genomic or cDNA), but are preferably synthetic (e.g. produced by oligonucleotide synthesis).

Exemplary immunostimulatory nucleic acids as those described herein as well as various control nucleic acids include but are not limited to those presented in Table 1.

**Table 1**

| SEQ ID NO: | ODN SEQUENCE              | BACKBONE |
|------------|---------------------------|----------|
| 1          | tctcccagcgtgcgccat        | s        |
| 2          | ataatccagcttgaaccaag      | s        |
| 3          | ataatcgacgttcaagcaag      | s        |
| 4          | taccgcggtgcgacctct        | s        |
| 5          | ggggaggggt                | s        |
| 6          | ggggagggg                 | s        |
| 7          | ggtgaggtg                 | s        |
| 8          | tccatgtzgttctctgatgct     | o        |
| 9          | gctaccttagzgtga           | o        |
| 10         | tccatgazgttctctgatgct     | o        |
| 11         | tccatgacgttctctgatgct     | o        |
| 12         | gctagazgttagtgt           | o        |
| 13         | agctccatggtgctcactg       | s        |
| 14         | ccacgtcgacctcaggcga       | s        |
| 15         | gcacatcgctcccgagccga      | s        |
| 16         | gtcaactcggtacctcga        | s        |
| 17         | gttggatacaggccagactttgttg | o        |
| 18         | gattcaacttgctcatcttaggc   | o        |
| 19         | accatggacgaactgtttccctc   | s        |
| 20         | accatggacgagctgtttccctc   | s        |
| 21         | accatggacgacctgtttccctc   | s        |
| 22         | accatggacgtactgtttccctc   | s        |
| 23         | accatggacggtctgtttccctc   | s        |
| 24         | accatggacgttctgtttccctc   | s        |
| 25         | ccactcacatctgctgctccacaag | o        |
| 26         | acttctcatagtccttttggtccag | o        |
| 27         | tccatgagcttctctgagtct     | o        |
| 28         | gaggaaggigiggaigacgt      | o        |
| 29         | gtgaaticgttctcgggict      | o        |
| 30         | aaaaaa                    | s        |
| 31         | ccccc                     | s        |
| 32         | ctgtca                    | s        |
| 33         | togtag                    | s        |

|    |                                         |     |
|----|-----------------------------------------|-----|
| 34 | togtgg                                  | s   |
| 35 | ogtctg                                  | s   |
| 36 | tccatgtcgggcctgagtct                    | sos |
| 37 | tccatgcgggcctgagtct                     | sos |
| 38 | tccatgacgggcctgagtct                    | sos |
| 39 | tccatgacgggcctgagtct                    | sos |
| 40 | tccatgtcgatcctgagtct                    | sos |
| 41 | tccatgtcgctcctgagtct                    | sos |
| 42 | tccatgtcgctcctgagtct                    | sos |
| 43 | tccatgacgctcctgagtct                    | sos |
| 44 | tccataacgctcctgagtct                    | sos |
| 45 | tccatgacgctcctgagtct                    | sos |
| 46 | tccatcacgtgcctgagtct                    | sos |
| 47 | tccatgctgggcctgagtct                    | sos |
| 48 | tccatgtzgggcctgagtct                    | sos |
| 49 | ccgttctctccagatgagctcatgggtttctccaccaag | o   |
| 50 | cttggtggagaaacccatgagctcatctggaggaagcgg | o   |
| 51 | cccaaagggatgagaagtt                     | o   |
| 52 | agatagcaaatacggtgacg                    | o   |
| 53 | ggttcacgtgctcatggctg                    | o   |
| 54 | tctcccagcgtgcgccat                      | s   |
| 55 | tctcccagcgtgcgccat                      | s   |
| 56 | taccgcgtgcgacctct                       | s   |
| 57 | ataatccagcttgaaccaag                    | s   |
| 58 | ataatcgacgttcaagcaag                    | s   |
| 59 | tccatgattttcctgatttt                    | o   |
| 60 | ttgtttttttgtttttttgttttt                | s   |
| 61 | ttttttttgtttttttgttttt                  | o   |
| 62 | tgctgctttttgtgctttttgtgctt              | s   |
| 63 | tgctgctttgtgctttttgtgctt                | o   |
| 64 | gcattcatcaggcgggcaagaat                 | o   |
| 65 | taccgagcttcgacgagatttca                 | o   |
| 66 | gcatgacgttgagct                         | s   |
| 67 | cacgttgaggggcat                         | s   |
| 68 | ctgctgagactggag                         | s   |
| 69 | tccatgacgttcctgacgtt                    | s   |
| 70 | gcatgagcttgagctga                       | o   |
| 71 | tcagcgtgcgcc                            | s   |
| 72 | atgacgttcctgacgtt                       | s   |
| 73 | ttttgggggttttgggggtttt                  | s   |
| 74 | tctaggctttttaggcttcc                    | s   |
| 75 | tgcattttttaggccaccat                    | s   |
| 76 | tctcccagcgtgcgtgcgccat                  | s   |
| 77 | tctcccagcggggcgcac                      | s   |
| 78 | tctcccagcgagcgccat                      | s   |
| 79 | tctcccagcgcgcgccat                      | s   |
| 80 | ggggtgacgttcagggggg                     | sos |
| 81 | ggggtccagcgtgcgccatggggg                | sos |
| 82 | ggggtgtcggttcagggggg                    | sos |
| 83 | tccatgtcggttcctgtcggt                   | s   |
| 84 | tccatagcgttcctagcgtt                    | s   |
| 85 | tcgtcgctgtctccgcttctt                   | s   |

|     |                              |     |
|-----|------------------------------|-----|
| 86  | gcatgacgttgagct              | sos |
| 87  | tctcccagcgtgcgccatat         | sos |
| 88  | tccatgazgttcctgazgtt         | s   |
| 89  | gcatgazgttgagct              | o   |
| 90  | tccagcgtgcgccata             | sos |
| 91  | tctcccagcgtgcgccat           | o   |
| 92  | tccatgagcttcctgagtct         | o   |
| 93  | gcatgtcgttgagct              | sos |
| 94  | tcctgacgttcctgacgtt          | s   |
| 95  | gcatgatgttgagct              | o   |
| 96  | gcatttcgaggagct              | o   |
| 97  | gcatgtagctgagct              | o   |
| 98  | tccaggacgttcctagttct         | o   |
| 99  | tccaggagcttcctagttct         | o   |
| 100 | tccaggatgttcctagttct         | o   |
| 101 | tccagtctaggcctagttct         | o   |
| 102 | tccagttcgagcctagttct         | o   |
| 103 | gcatggcgttgagct              | sos |
| 104 | gcatagcgttgagct              | sos |
| 105 | gcattgcgttgagct              | sos |
| 106 | gcttgcggttgagct              | sos |
| 107 | tctcccagcgttgcccatat         | sos |
| 108 | tctcccagcgttgcttatat         | sos |
| 109 | tctccctgcgtgcgccatat         | sos |
| 110 | tctgcgtgcgtgcgccatat         | sos |
| 111 | tctcctagcgtgcgccatat         | sos |
| 112 | tctcccagcgtgcgcctttt         | sos |
| 113 | gctandcghhagc                | o   |
| 114 | tcctgacgttccc                | o   |
| 115 | ggaagacgttaga                | o   |
| 116 | tcctgacgttaga                | o   |
| 117 | tcagaccagctggctgggtgttcctga  | o   |
| 118 | tcaggaacacccgaccagctggctctga | o   |
| 119 | gctagtcgatagc                | o   |
| 120 | gctagtcgctagc                | o   |
| 121 | gcttgacgtctagc               | o   |
| 122 | gcttgacgttttagc              | o   |
| 123 | gcttgacgtcaagc               | o   |
| 124 | gctagacgttttagc              | o   |
| 125 | tccatgacattcctgatgct         | o   |
| 126 | gctagacgtctagc               | o   |
| 127 | ggctatgtcgttcctagcc          | o   |
| 128 | ggctatgtcgatcctagcc          | o   |
| 129 | ctcatgggtttctccaccaag        | o   |
| 130 | cttgggtggagaaacccatgag       | o   |
| 131 | tccatgacgttcctagttct         | o   |
| 132 | ccgcttcctccagatgagctcatg     | o   |
| 133 | catgagctcatctggaggaagcgg     | o   |
| 134 | ccagatgagctcatgggtttctcc     | o   |
| 135 | ggagaaacccatgagctcatctgg     | o   |
| 136 | agcatcaggaacgacatgga         | o   |
| 137 | tccatgacgttcctgacgtt         | rna |

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|     |                                           |          |
|-----|-------------------------------------------|----------|
| 190 | tcgagacattgcacaatcatctg                   | o        |
| 191 | cagattgtgcaatgtctcga                      | o        |
| 192 | tccatgtcggttcctgatgcg                     | o        |
| 193 | gcgatgtcggttcctgatgct                     | o        |
| 194 | gcgatgtcggttcctgatgcg                     | o        |
| 195 | tccatgtcggttcgcgcgcgcg                    | o        |
| 196 | tccatgtcggttcctgcccgt                     | o        |
| 197 | tccatgtcggttcctgtagct                     | o        |
| 198 | gcggcgggcggcgcgcgcgc                      | o        |
| 199 | atcaggaacgtcatgggaagc                     | o        |
| 200 | tccatgagcttcctgagctc                      | p-ethoxy |
| 201 | tcaacggt                                  | p-ethoxy |
| 202 | tcaagctt                                  | p-ethoxy |
| 203 | tcctgtcggttcctgtcggt                      | s        |
| 204 | tccatgtcggtttttgtcggt                     | s        |
| 205 | tcctgtcggttcctgtcggt                      | s        |
| 206 | tcctgtcggttcctgtcggt                      | s        |
| 207 | btccattccatgacgttcctgatgcttcca            | os       |
| 208 | tcctgtcggtttttgtcggt                      | s        |
| 209 | tcgtcgctgtctccgcttctt                     | s        |
| 210 | tcgtcgctgtctcccttctt                      | s        |
| 211 | tcgtcgctgttgcgtttctt                      | s        |
| 212 | tcctgtcggttcctgtcggttggaaacgacagg         | o        |
| 213 | tcctgtcggttcctgtcggttcaacgtcaggaacgacagga | o        |
| 214 | gggggtctgtcggttttgggggg                   | sos      |
| 215 | gggggtctgtcggttttgggggg                   | sos      |
| 216 | tccggcggttgaagt                           | o        |
| 217 | tccggacggtgaagt                           | o        |
| 218 | tcccgcggttgaagt                           | o        |
| 219 | tccagacggtgaagt                           | o        |
| 220 | tcccgcggttgaagt                           | o        |
| 221 | tccagagcttgaagt                           | o        |
| 222 | tccatgtzgttcctgtzgtt                      | s        |
| 223 | tccatgacgttcctgacgtt                      | sos      |
| 224 | gggggttgcggttttgggggg                     | sos      |
| 225 | tccaggacttctctcaggtt                      | s        |
| 226 | tttttttttttttttttttt                      | s        |
| 227 | tccatgccgttcctgccgtt                      | s        |
| 228 | tccatggcgggcctggcggg                      | s        |
| 229 | tccatgacgttcctgccgtt                      | s        |
| 230 | tccatgacgttcctggcggg                      | s        |
| 231 | tccatgacgttcctgccgtt                      | s        |
| 232 | tccatgacggtcctgacggt                      | s        |
| 233 | tccatgcgtgcgtgcgtttt                      | s        |
| 234 | tccatgcgttgcgttgcgtt                      | s        |
| 235 | btccattccattctaggcctgagcttccat            | os       |
| 236 | tccatagcgttcctagcgtt                      | o        |
| 237 | tccatgtcggttcctgtcggt                     | o        |
| 238 | tccatagcgatcctagcgat                      | o        |
| 239 | tccattgcgttccttgcgtt                      | o        |
| 240 | tccatagcggtcctagcggt                      | o        |
| 241 | tccatgattttctgcagttcctgat                 | o        |



|     |                                          |     |
|-----|------------------------------------------|-----|
| 242 | tccatgacgttcctgcagttcctgacgtt            | s   |
| 243 | ggcgggcgggcgggcgggcg                     | o   |
| 244 | tccacgacgttttcgacgtt                     | s   |
| 245 | tcgtcgttgtcgttgtcggt                     | s   |
| 246 | tcgtcgttttgtcgttttgtcggt                 | s   |
| 247 | tcgtcgttgtcgttttgtcggt                   | s   |
| 248 | gcgtgcgttgtcgttgtcggt                    | s   |
| 249 | czggczggczggczggczgg                     | o   |
| 250 | gcggcgggcgggcgggcgccc                    | s   |
| 251 | agicccgigaacgiattcac                     | o   |
| 252 | tgtcgtttgtcgtttgtcggt                    | s   |
| 253 | tgtcgttgtcgttgtcggtgtcggt                | s   |
| 254 | tgtcgttgtcgttgtcggtgtcggt                | s   |
| 255 | tcgtcgtcgtcggt                           | s   |
| 256 | tgtcgttgtcggt                            | s   |
| 257 | cccccccccccccccccc                       | s   |
| 258 | tctagcgtttttagcggtcc                     | sos |
| 259 | tgcacccccagccaccat                       | s   |
| 260 | tcgtcgtcgtcgtcgtcgtcgtt                  | sos |
| 261 | tcgtcgttgtcgttgtcggt                     | sos |
| 262 | tcgtcgttttgtcgttttgtcggt                 | sos |
| 263 | tcgtcgttgtcgttttgtcggt                   | sos |
| 264 | ggggaggagggaacttcctaaaattccccagaatgttt   | o   |
| 265 | aaacattctgggggaatttttaagaagtccctccctcccc | o   |
| 266 | atgtttacttcctaaaattccccagaatgttt         | o   |
| 267 | aaacattctgggggaatttttaagaagtaaacat       | o   |
| 268 | atgtttactagacaaaattccccagaatgttt         | o   |
| 269 | aaacattctgggggaattttgtctagtaaacat        | o   |
| 270 | aaaattgacgttttaaaaaa                     | sos |
| 271 | cccttgacgttttcccccc                      | sos |
| 272 | tttgcgttgttttgcgtt                       |     |
| 273 | tcgtcgtttgcgttttgcgtt                    | sos |
| 274 | ctgcagcctgggac                           | o   |
| 275 | accgctcgtaattatagtaaaaccc                | o   |
| 276 | ggtacctgtggggacattgtg                    | o   |
| 277 | agcaccgaacgtgagagg                       | o   |
| 278 | tccatgccgttcctgccgtt                     | o   |
| 279 | tccatgacggtcctgacggt                     | o   |
| 280 | tccatgccgttcctgccgtt                     | o   |
| 281 | tccatgcgcgttcctgcgcgt                    | o   |
| 282 | ctggtctttctggttttttctgg                  | s   |
| 283 | tcaggggtgggggaacctt                      | sos |
| 284 | tccatgazgttcctagttct                     | o   |
| 285 | tccatgatgttcctagttct                     | o   |
| 286 | cccgaagtcatttcctcttaacctgg               | o   |
| 287 | ccaggttaagaggaaatgacttcggg               | o   |
| 288 | tcctggzggggaagt                          | o   |
| 289 | gzggzgggzggzgzgzgccc                     | x   |
| 290 | tccatgtgcttcctgatgct                     | o   |
| 291 | tccatgtccttcctgatgct                     |     |
| 292 | tccatgtcgttcctagttct                     |     |
| 293 | tccaagtagttcctagttct                     | o   |

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|     |                      |     |
|-----|----------------------|-----|
| 294 | tccatgtagttcctagttct | o   |
| 295 | tcccgcgcggttccgcggtt | s   |
| 296 | tcccgcgcggttccgcggtt | s   |
| 297 | tcccgcgcggttccgcggtt | o   |
| 298 | tcccgcgcggttccgcggtt | o   |
| 299 | tcccgcgcggttccgcggtt | o   |
| 300 | tcccgcgcggttccgcggtt | o   |
| 301 | tcccgcgcggttccgcggtt | o   |
| 302 | tcccgcgcggttccgcggtt | o   |
| 303 | tcccgcgcggttccgcggtt | sos |
| 304 | tcccgcgcggttccgcggtt | o   |
| 305 | tcccgcgcggttccgcggtt | os  |
| 306 | tcccgcgcggttccgcggtt | s   |
| 307 | tcccgcgcggttccgcggtt | s   |
| 308 | tcccgcgcggttccgcggtt | o   |
| 309 | tcccgcgcggttccgcggtt | s   |
| 310 | tcccgcgcggttccgcggtt | s   |
| 311 | tcccgcgcggttccgcggtt | s   |
| 312 | tcccgcgcggttccgcggtt | s   |
| 313 | tcccgcgcggttccgcggtt | s   |
| 314 | tcccgcgcggttccgcggtt | o   |
| 315 | tcccgcgcggttccgcggtt | o   |
| 316 | tcccgcgcggttccgcggtt | os  |
| 317 | tcccgcgcggttccgcggtt | o   |
| 318 | tcccgcgcggttccgcggtt | o   |
| 319 | tcccgcgcggttccgcggtt | o   |
| 320 | tcccgcgcggttccgcggtt | o   |
| 321 | tcccgcgcggttccgcggtt | o   |
| 322 | tcccgcgcggttccgcggtt | o   |
| 323 | tcccgcgcggttccgcggtt | o   |
| 324 | tcccgcgcggttccgcggtt | o   |
| 325 | tcccgcgcggttccgcggtt | s   |
| 326 | tcccgcgcggttccgcggtt | s   |
| 327 | tcccgcgcggttccgcggtt | s   |
| 328 | tcccgcgcggttccgcggtt | s   |
| 329 | tcccgcgcggttccgcggtt | s   |
| 330 | tcccgcgcggttccgcggtt | sos |
| 331 | tcccgcgcggttccgcggtt | o   |
| 332 | tcccgcgcggttccgcggtt | o   |
| 333 | tcccgcgcggttccgcggtt | o   |
| 334 | tcccgcgcggttccgcggtt | o   |
| 335 | tcccgcgcggttccgcggtt | o   |
| 336 | tcccgcgcggttccgcggtt | o   |
| 337 | tcccgcgcggttccgcggtt | o   |
| 338 | tcccgcgcggttccgcggtt | o   |
| 339 | tcccgcgcggttccgcggtt | o   |
| 340 | tcccgcgcggttccgcggtt | o   |
| 341 | tcccgcgcggttccgcggtt | o   |
| 342 | tcccgcgcggttccgcggtt | o   |
| 343 | tcccgcgcggttccgcggtt | s   |
| 344 | tcccgcgcggttccgcggtt | s   |
| 345 | tcccgcgcggttccgcggtt | o   |

|     |                                       |     |
|-----|---------------------------------------|-----|
| 346 | gcgtcggtccccccccccc                   | o   |
| 347 | tcgtcattccccccccccc                   | o   |
| 348 | acgtcggtccccccccccc                   | o   |
| 349 | ctgtcggtccccccccccc                   | o   |
| 350 | btttttcgtcggtccccccccccc              | os  |
| 351 | tcgtcggtcccccccccccb                  | o   |
| 352 | tcgtcggtttgtcggtttgtcggtb             | o   |
| 353 | tccagttccttctcagttct                  | o   |
| 354 | tzgtcggtttgtcggtttgtcggt              | o   |
| 355 | tcctggaggggaagt                       | s   |
| 356 | tcctgaaaaggaagt                       | s   |
| 357 | tcgtcggtcccccccccc                    | s   |
| 358 | tzgtzgtttgtzgtttgtzgtt                | sos |
| 359 | ggggtcaagcttgagggggg                  | s   |
| 360 | tgctgcttccccccccccc                   | s2  |
| 361 | tcgtcggtcggt                          | s20 |
| 362 | tcgtcggtcggt                          | os2 |
| 363 | tcgtcggtcggt                          | s   |
| 364 | tcaacgttga                            | s   |
| 365 | tcaacgtt                              |     |
| 366 | atagttttccatttttttac                  |     |
| 367 | aatagtcgcatcgcgagac                   | o   |
| 368 | aatagtcgcatcccgagac                   | o   |
| 369 | aatagtcgcatccccccc                    | o   |
| 370 | tgctgctttgtgctttgtgctt                | o   |
| 371 | ctgtgctttctgtgtttttctgtg              | s   |
| 372 | ctaacttttctaatttttttctaa              | s   |
| 373 | tcgtcggttggtgctgttggtgctgt            | s   |
| 374 | tcgtcggttggtgctgttttggtt              | s   |
| 375 | accatggacgagctgttccctc                |     |
| 376 | tcgtcggtttgctgctgtt                   | s   |
| 377 | ctgtaagtgagcttgagag                   |     |
| 378 | gagaacgctggaccttc                     |     |
| 379 | cgggcgactcagttctatcg                  |     |
| 380 | gttctcagataaagcggaaccagcaacagacacagaa |     |
| 381 | ttctgtgtctgttgctgggttcgctttatctgagaac |     |
| 382 | cagacacagaagccgatagacg                |     |
| 383 | agacagacacgaaacgaccg                  |     |
| 384 | gtctgtcccatgatctcgaa                  |     |
| 385 | gctggccagcttacctcccg                  |     |
| 386 | ggggcctctatacaacctggg                 |     |
| 387 | ggggtccctgagactgcc                    |     |
| 388 | gagaacgctggaccttccat                  |     |
| 389 | tccatgtcggctctgatgt                   |     |
| 390 | ctcttgcgacctggaagta                   |     |
| 391 | aggtacagccaggactacga                  |     |
| 392 | accatggacgacctgttccctc                |     |
| 393 | accatggattacctttttccctt               |     |
| 394 | atggaagggtccagcgttctc                 | o   |
| 395 | agcatcaggaccgacatgga                  | o   |
| 396 | ctctccaagctcacttacag                  |     |
| 397 | tccttgagactgccccacctt                 |     |

|     |                                      |     |
|-----|--------------------------------------|-----|
| 398 | gccacaaaaacttgatccatg                |     |
| 399 | gtccatggcgtgcgggatga                 |     |
| 400 | cctctatacaacctgggac                  |     |
| 401 | cgggcgactcagtctatcgg                 |     |
| 402 | gcgctaccggtagcctgagt                 |     |
| 403 | cgactgccgaacaggatatcggtgatcagcactgg  |     |
| 404 | ccagtgcctgatcaccgatatcctgttcggcagtcg |     |
| 405 | ccagggttgatatagaggc                  |     |
| 406 | tctcccagcgtacgccat                   | S   |
| 407 | tctcccagcgtgcgtttt                   | S   |
| 408 | tctcccagcgtgcgccat                   | S   |
| 409 | tctcccgtcgtgcgccat                   | S   |
| 410 | ataatcgctcgttcaagcaag                | S   |
| 411 | tcgtcgttttgtcgttttgtcgt              | S2  |
| 412 | tcgtcgttttgtcgttttgtcgtt             | S2  |
| 413 | tcgtcgttttgtcgttttgtcgtt             | S2  |
| 414 | tctcgctnttntcgctnttntcgtn            | S   |
| 415 | tctcccagcgtcgccat                    | S   |
| 416 | tctcccagcgtcgccat                    | S   |
| 417 | ataatcgctcgttcaagaaag                | S   |
| 418 | ataatcgacgttcccccccc                 | S   |
| 419 | tctatcgacgttcaagcaag                 | S   |
| 420 | tcc tga cgg gg agt                   | S   |
| 421 | tccatgacgttcctgatcc                  |     |
| 422 | tccatgacgttcctgatcc                  |     |
| 423 | tccatgacgttcctgatcc                  |     |
| 424 | tcc tgg cgt gga agt                  | S   |
| 425 | tccatgacgttcctgatcc                  |     |
| 426 | tcgtcgctgttgcgttttctt                | S   |
| 427 | agcagcttagagcttagagctt               | S   |
| 428 | cccccccccccccccccccc                 | S   |
| 429 | tcgtcgttttgtcgttttgtcgttttgtcgtt     | S   |
| 430 | tcgtcgttttgtcgttttgtcgtt             | S   |
| 431 | tcgtcgttttgtcgttttgtcgtt             | S   |
| 432 | tttttcaacgttgatttttt                 | SOS |
| 433 | tttttttttttttttttttt                 | S   |
| 434 | ggggtcgctcgttttggggg                 |     |
| 435 | tcgtcgttttgtcgttttggggg              |     |
| 436 | tcgtcgctgtctccgcttcttcttgc           | S   |
| 437 | tcgtcgctgtctccg                      | S   |
| 438 | ctgtaagtgcgttgagag                   |     |
| 439 | gagaacgctggaccttccat                 |     |
| 440 | ccagggttgatatagaggc                  |     |
| 441 | gctagacgttagcgtga                    |     |
| 442 | ggagctcttcgaacgccata                 |     |
| 443 | tctccatgatggttttatcg                 |     |
| 444 | aagggtggggcagtcctcaggga              |     |
| 445 | atcgaggactggcgcgccg                  |     |
| 446 | ttaggacaaggctcagggtg                 |     |
| 447 | accacaacgagaggaacgca                 |     |
| 448 | ggcagtgacaggtcaccggg                 |     |
| 449 | gaaccttccatgctgtt                    |     |

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|     |                                  |     |
|-----|----------------------------------|-----|
| 450 | gctagacgttagcgtga                |     |
| 451 | gcttggagggcctgtaagt              |     |
| 452 | gtagccttccta                     |     |
| 453 | cggtagccttccta                   |     |
| 454 | caaggtagccttccta                 |     |
| 455 | agcacggtagccttccta               |     |
| 456 | gaacgctggaccttccat               |     |
| 457 | gaccttccat                       |     |
| 458 | tggaccttccat                     |     |
| 459 | gctggaccttccat                   |     |
| 460 | acgctggaccttccat                 |     |
| 461 | taagctctgtcaacgccagg             |     |
| 462 | gagaacgctggaccttccatgt           |     |
| 463 | tccatgtcggctcctgatgct            |     |
| 464 | ttcatgccttgcaaaatggcg            |     |
| 465 | tgctagctgtgcctgtacct             |     |
| 466 | agcatcaggaccgacatgga             |     |
| 467 | gaccttccatgtcggctcctgat          |     |
| 468 | acaaccacgagaacgggaac             |     |
| 469 | gaaccttccatgctgttccg             |     |
| 470 | caatcaatctgaggagaccc             |     |
| 471 | tcagctctggactttttca              |     |
| 472 | tggttacggctctgtcccatg            |     |
| 473 | gtctatcgaggactggcgc              |     |
| 474 | cattttacggcgggcgggc              |     |
| 475 | gaggggaccattttacgggc             |     |
| 476 | tgtccagccgaggggaccat             |     |
| 477 | cgggcttacggcggatgctg             |     |
| 478 | tggaccttctatgtcggctc             |     |
| 479 | tgtcccatgtttttagaagc             |     |
| 480 | gtggttacggctcgtgcccat            |     |
| 481 | cctccaaatgaaagaccccc             |     |
| 482 | ttgtactctccatgatggtt             |     |
| 483 | ttccatgctgttccgctgg              |     |
| 484 | gaccttctatgtcggctcctg            |     |
| 485 | gagaccgctcgaccttcgat             |     |
| 486 | ttgccccatattttagaaac             |     |
| 487 | ttgaaactgaggtgggac               |     |
| 488 | ctatcggaggactggcgcgcc            |     |
| 489 | cttggagggcctcccggcg              |     |
| 490 | gctgaaccttccatgctgtt             |     |
| 491 | tagaaacagcattcttcttttagggcagcaca |     |
| 492 | agatggttctcagataaagcgaa          |     |
| 493 | ttccgctttatctgagaaccatct         |     |
| 494 | gtcccaggttgatagaggctgc           |     |
| 495 | gcgccagtccctcgatagac             |     |
| 496 | atcggaggactggcgcgccg             |     |
| 497 | ggtctgtcccatatttttag             |     |
| 498 | tttttcaacgttgagggggg             | SOS |
| 499 | tttttcaagcgttgatttttt            | SOS |
| 500 | ggggtcaacgttgatttttt             | SOS |
| 501 | ggggttttcaacgttttgagggggg        | SOS |

|     |                                      |     |
|-----|--------------------------------------|-----|
| 502 | ggttacggtctgtcccatat                 |     |
| 503 | ctgtcccatatttttagaca                 |     |
| 504 | accatcctgaggccattcgg                 |     |
| 505 | cgtctatcgggcttctgtgtctg              |     |
| 506 | ggccatcccacattgaaagtt                |     |
| 507 | ccaaatatcggtggtcaagcac               |     |
| 508 | gtgcttgaccaccgataatttg               |     |
| 509 | gtgctgatcaccgatacctgttcgg            |     |
| 510 | ggccaactttcaatgtgggatggctc           |     |
| 511 | ttccgccgaatggcctcaggatggtac          |     |
| 512 | tatagtccctgagactgccccaccttctcaacaacc |     |
| 513 | gcagcctctatacaacctgggacggga          |     |
| 514 | ctatcggaggactggcgcgccg               |     |
| 515 | tatcggaggactggcgcgccg                |     |
| 516 | gatcggaggactggcgcgccg                |     |
| 517 | ccgaacaggatatcggtgatcagcac           |     |
| 518 | ttttggggtcaacgttgagggggg             |     |
| 519 | gggggtcaacgttgagggggg                | sos |
| 520 | cgcgcgcgcgcgcgcgcgcgcg               | s   |
| 521 | ggggcatgacgttcgggggg                 | ss  |
| 522 | ggggcatgacgttcaaaaaa                 | s   |
| 523 | ggggcatgacgttcgggggg                 | s   |
| 524 | ggggcatgacgttcgggggg                 | sos |
| 525 | aaaacatgacgttcaaaaaa                 | sos |
| 526 | aaaacatgacgttcgggggg                 | sos |
| 527 | ggggcatgacgttcaaaaaa                 | sos |
| 528 | accatggacgatctgtttcccctc             | s   |
| 529 | gccatggacgaactgtttcccctc             | s   |
| 530 | cccccccccccccccccccc                 | sos |
| 531 | ggggggggggggggggggggg                | sos |
| 532 | gctgtaaaatgaatcggccg                 | sos |
| 533 | ttcgggcggactcctccatt                 | sos |
| 534 | tatgccgcgcccggaacttat                | sos |
| 535 | ggggtaatcgatcagggggg                 | sos |
| 536 | tttgagaacgctggaccttc                 | sos |
| 537 | gatcgtgatctaatactctg                 | sos |
| 538 | gtcgggtcctgatgctgttcc                | sos |
| 539 | tcgtcgtcagttcgtgtctg                 | sos |
| 540 | ctggaccttccatgtcgg                   | sos |
| 541 | gctcgttcagcgcgtct                    | sos |
| 542 | ctggaccttccatgtc                     | sos |
| 543 | caactgtccttcgtcga                    | sos |
| 544 | cgtcggaccttccatgtcgg                 | sos |
| 545 | gctgagctcatgccgtctgc                 | sos |
| 546 | aacgctggaccttccatgtc                 | sos |
| 547 | tgcattgccgtacacagctct                | sos |
| 548 | ccttccatgtcgggtcctgat                | sos |
| 549 | tactcttcggatcccttgcg                 | sos |
| 550 | ttccatgtcgggtcctgat                  | sos |
| 551 | ctgattgctctctcgtga                   | sos |
| 552 | ggcgttattcctgactcgcc                 | o   |
| 553 | cctacgttgatgcgccagct                 | o   |

|     |                                               |   |
|-----|-----------------------------------------------|---|
| 554 | ggggtaatcgatgagggggg                          | o |
| 555 | ttcgggcggactcctccatt                          | o |
| 556 | tttttttttttttttttttt                          | o |
| 557 | gggggttttttttttggggg                          | o |
| 558 | tttttgggggggggttttt                           | o |
| 559 | gggggggggggggggggt                            | o |
| 560 | aaaaaaaaaaaaaaaaaaaa                          | o |
| 561 | cccccaaaaaaaaaaccccc                          | o |
| 562 | aaaaaccccccccccaaaaa                          | o |
| 563 | tttgaattcaggactggtgaggttgag                   | o |
| 564 | tttgaatcctcagcgggtctccagtggc                  | o |
| 565 | aattctctatcggggcttctgtgtctgttgctgggtccgctttat | o |
| 566 | ctagataaagcggaaccagcaacagacacagaagccccgatagag | o |
| 567 | ttttctagagaggtgcacaatgctctgg                  | o |
| 568 | tttgaattccgtgtacagaagcgagaagc                 | o |
| 569 | tttgcggccgctagacttaacctgagagata               | o |
| 570 | tttggggccacgagagacagagacacttc                 | o |
| 571 | tttggggccgcttctcgcttctgtacacg                 | o |
| 572 | gagaacgctggaccttccat                          | s |
| 573 | tccatgtcggctcctgatgct                         | s |
| 574 | ctgtcg                                        | s |
| 575 | tcgtga                                        | s |
| 576 | cgtcga                                        | s |
| 577 | agtgc                                         | s |
| 578 | ctgtcg                                        | o |
| 579 | agtgc                                         | o |
| 580 | cgtcga                                        | o |
| 581 | tcgtga                                        | o |
| 582 | gagaacgctccagcttcgat                          | o |
| 583 | gctagacgtaagcgtga                             | o |
| 584 | gagaacgctcgaccttccat                          | o |
| 585 | gagaacgctggacctatccat                         | o |
| 586 | gctagagggttagcgtga                            | o |
| 587 | gagaacgctggaccttccat                          | o |
| 588 | tcacgctaacgtctagc                             | o |
| 589 | bgctagacggttagcgtga                           | o |
| 590 | atggaaggtcgagcgttctc                          | o |
| 591 | gagaacgctggaccttcgat                          | o |
| 592 | gagaacgatggaccttccat                          | o |
| 593 | gagaacgctggatccat                             | o |
| 594 | gagaacgctccagcactgat                          | o |
| 595 | tccatgtcggctcctgatgat                         | o |
| 596 | atgtcctcggctcctgatgct                         | o |
| 597 | gagaacgctccaccttccat                          | o |
| 598 | gagaacgctggaccttcgta                          | o |
| 599 | batggaaggtccagcgttctc                         | o |
| 600 | tcctga                                        | o |
| 601 | tcaacggt                                      | o |
| 602 | aacggt                                        | o |
| 603 | aacggtga                                      | o |
| 604 | tcacgctaacctctagc                             | o |
| 605 | gagaacgctggaccttgc                            | o |

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|-----|-------------------------|---|
| 606 | gctggaccttccat          | o |
| 607 | gagaacgctggacctcatccat  | o |
| 608 | gagaacgctggacgctcatccat | o |
| 609 | aacgttgaggggcat         | o |
| 610 | atgccctcaacgtt          | o |
| 611 | tcaacgttga              | o |
| 612 | gctggaccttccat          | o |
| 613 | caacgtt                 | o |
| 614 | acaacgttga              | o |
| 615 | tcacgt                  | o |
| 616 | tcaagctt                | o |
| 617 | tcgtca                  | o |
| 618 | aggatatac               | o |
| 619 | tagacgtc                | o |
| 620 | gacgtcat                | o |
| 621 | ccatcgat                | o |
| 622 | atcgatgt                | o |
| 623 | atgcatgt                | o |
| 624 | ccatgcat                | o |
| 625 | agcgctga                | o |
| 626 | tcagcgct                | o |
| 627 | ccttcgat                | o |
| 628 | gtgccgggggtctccgggc     | s |
| 629 | gctgtggggcggtcctg       | s |
| 630 | btcaacgtt               | o |
| 631 | ftcaacgtt               | o |
| 632 | faacgttga               | o |
| 633 | tcaacgt                 | s |
| 634 | aacgttg                 | s |
| 635 | cgacga                  | o |
| 636 | tcaacgtt                | o |
| 637 | tcgga                   | o |
| 638 | agaacgtt                | o |
| 639 | tcacgat                 | o |
| 640 | taaacgtt                | s |
| 641 | ccaacgtt                | s |
| 642 | gctcga                  | s |
| 643 | cgacgt                  | s |
| 644 | cgtcgt                  | s |
| 645 | acgtgt                  | s |
| 646 | cgttcg                  | s |
| 647 | gagcaagctggaccttccat    | s |
| 648 | cgcgta                  | s |
| 649 | cgtaag                  | s |
| 650 | tcacgggt                | s |
| 651 | caagagatgctaacaatgca    | s |
| 652 | acccatcaatagctctgtgc    | s |
| 653 | ccatcgat                | o |
| 654 | tcgacgtc                | o |
| 655 | ctagcgct                | o |
| 656 | taagcgct                | o |
| 657 | tcgcgaattcgcg           | o |



|     |                           |   |
|-----|---------------------------|---|
| 658 | atggaaggtccagcgttct       | o |
| 659 | actggacgttagcgtga         | o |
| 660 | cgcctggggctggtctgg        | o |
| 661 | gtgtcggggctcgcgggc        | o |
| 662 | gtgccggggctcgcgggc        | o |
| 663 | cgcgctcgcggcggttg         | o |
| 664 | gaagttcacgttgaggggcat     | o |
| 665 | atctggtgagggcaagctatg     | s |
| 666 | gttgaaacccgagaacatcat     | s |
| 667 | gcaacgtt                  | o |
| 668 | gtaacgtt                  | o |
| 669 | cgaacgtt                  | o |
| 670 | gaaacgtt                  | o |
| 671 | caaacgtt                  | o |
| 672 | ctaacgtt                  | o |
| 673 | ggaacgtt                  | o |
| 674 | tgaacgtt                  | o |
| 675 | acaacgtt                  | o |
| 676 | ttaacgtt                  | o |
| 677 | aaaacgtt                  | o |
| 678 | ataacgtt                  | o |
| 679 | aacgttct                  | o |
| 680 | tccgatcg                  | o |
| 681 | tccgtacg                  | o |
| 682 | gctagacgctagcgtga         | o |
| 683 | gagaacgctggacctcatcatccat | o |
| 684 | gagaacgctagaccttctat      | o |
| 685 | actagacgttagtgtga         | o |
| 686 | cacaccttggtcaatgtcacgt    | o |
| 687 | tctccatcctatggttttatcg    | o |
| 688 | cgtgacaccttccat           | o |
| 689 | caccaccttggtcaatgtcacgt   | o |
| 690 | gctagacgttagctgga         | o |
| 691 | agtgcgattgcagatcg         | o |
| 692 | ttttcgttttgtggtttgtggtt   |   |
| 693 | ttttcgttttgtggtttgtcggt   |   |
| 694 | ttttgtttttgtggtttgtggtt   |   |
| 695 | accgcatggattctaggcca      | s |
| 696 | gctagacgttagcgt           | o |
| 697 | aacgctggaccttccat         | o |
| 698 | tcaazggt                  | o |
| 699 | ccttcgat                  | o |
| 700 | actagacgttagtgtga         | s |
| 701 | gctagaggttagcgtga         | s |
| 702 | atggactctccagcgttctc      | o |
| 703 | atcgactctcgagcgttctc      | o |
| 704 | gctagacgttagc             | o |
| 705 | gctagacgt                 | o |
| 706 | agtgcgattcgagatcg         | o |
| 707 | tcagzgct                  | o |
| 708 | ctgattgctctctcgtga        | o |
| 709 | tzaacgtt                  | o |

|     |                        |     |
|-----|------------------------|-----|
| 710 | gagaazgctggaccttccat   | o   |
| 711 | gctagacgcttaggctga     | o   |
| 712 | gctacttagcgtga         | o   |
| 713 | gctaccttagcgtga        | o   |
| 714 | atcgacttcgagcgttctc    | o   |
| 715 | atgcactctgcagcgttctc   | o   |
| 716 | agtgaactctccagcgttctc  | o   |
| 717 | gccagatgttagctgga      | o   |
| 718 | atcgactcgagcgttctc     | o   |
| 719 | atcgatcgagcgttctc      | o   |
| 720 | bgagaacgctcgaccttcgat  | o   |
| 721 | gctagacgcttagctgga     | SOS |
| 722 | atcgactctcgagcgttctc   | SOS |
| 723 | tagacgcttagcgtga       | o   |
| 724 | cgactctcgagcgttctc     | o   |
| 725 | ggggtcgaccttggagggggg  | SOS |
| 726 | gctaacgcttagcgtga      | o   |
| 727 | cgctcgtcgt             | o   |
| 728 | gagaacgctggacztccat    | o   |
| 729 | atcgacctacgtgcgttztc   | o   |
| 730 | atzgacctacgtgcgttctc   | o   |
| 731 | gctagazgttagcgt        | o   |
| 732 | atcgactctcgagzgttctc   | o   |
| 733 | ggggtaatgcatcagggggg   | SOS |
| 734 | ggctgtattcctgactgcc    | S   |
| 735 | ccatgctaacctctagc      | o   |
| 736 | gctagatgttagcgtga      | o   |
| 737 | cgtaccttacggtga        | o   |
| 738 | tccatgctggctcctgatgct  | o   |
| 739 | atcgactctctcgagcgttctc | o   |
| 740 | gctagagccttagcgtga     | o   |
| 741 | atcgactctcgagtgttctc   | o   |
| 742 | aacgctcgaccttcgat      | o   |
| 743 | ctcaacgctggaccttccat   | o   |
| 744 | atcgacctacgtgcgttctc   | o   |
| 745 | gagaatgctggaccttccat   | o   |
| 746 | tcacgctaacctctgac      | o   |
| 747 | bgagaacgctccagcactgat  | o   |
| 748 | bgagcaagctggaccttccat  | o   |
| 749 | cgctagaggcttagcgtga    | o   |
| 750 | gctagatgttaacgt        | o   |
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| 752 | gctagatgttagcgt        | o   |
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| 755 | tccatggcggctcctgatgct  | o   |
| 756 | gctagacgatagcgt        | o   |
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| 758 | tccatgacgttcctgatgct   | o   |
| 759 | tccatgtcgttcctgatgct   | o   |
| 760 | gctagacgttagzgt        | o   |
| 761 | gctaggcgttagcgt        | o   |

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|     |                                  |     |
|-----|----------------------------------|-----|
| 814 | tgcacacagctct                    | sos |
| 815 | tgcacacagctacacagctct            | o   |
| 816 | gagcaagctggaccttccat             | s   |
| 817 | tcaacgttaacgttaacgttaacgttaacgtt | s   |
| 818 | gagaacgctcgaccttcgat             | s   |
| 819 | gtccccatttcccagaggaggaaat        | o   |
| 820 | ctagcggctgacgtcatcaagctag        | o   |
| 821 | ctagcttgatgacgtcagccgctag        | o   |
| 822 | cggctgacgtcatcaa                 | s   |
| 823 | ctgacgtg                         | o   |
| 824 | ctgacgtcat                       | o   |
| 825 | attcgatcggggcggggcgag            | o   |
| 826 | ctcggcccgcccgatcgaat             | o   |
| 827 | gactgacgtcagcgt                  | o   |
| 828 | ctagcggctgacgtcataaagctagc       | s   |
| 829 | ctagctttatgacgtcagccgctagc       | s   |
| 830 | ctagcggctgagctcataaagctagc       | s   |
| 831 | ctagtggctgacgtcatcaagctag        | s   |
| 832 | tccaccacgtggtctatgct             | s   |
| 833 | gggaatgaaagattttattataag         | o   |
| 834 | tctaaaaaccatctattcttaaccct       | o   |
| 835 | agctcaacgtcatgc                  | o   |
| 836 | ttaacggtggttagcgggtattggtc       | o   |
| 837 | ttaagaccaataaccgctaccaccg        | o   |
| 838 | gatctagtgatgagtcagccggatc        | o   |
| 839 | gatccggctgactcatcactagatc        | o   |
| 840 | tccaagacgttcctgatgct             | o   |
| 841 | tccatgacgtccctgatgct             | o   |
| 842 | tccaccacgtggctgatgct             | o   |
| 843 | ccacgtggacctctagc                | o   |
| 844 | tcagaccacgtggtcgggtgttcctga      | o   |
| 845 | tcaggaacacccgaccacgtggtctga      | o   |
| 846 | catttccacgatttccca               | o   |
| 847 | ttcctctctgcaagagact              | o   |
| 848 | tgtatctctctgaaggact              | o   |
| 849 | ataaagcgaaactagcagcagtttc        | o   |
| 850 | gaaactgctgctagtttcgctttat        | o   |
| 851 | tgcccaaagaggaaaatttggttcatacag   | o   |
| 852 | ctgtatgaaacaaatttctctcttgggca    | o   |
| 853 | ttaggggttaggggttaggggt           | ss  |
| 854 | tccatgagcttcctgatgct             | ss  |
| 855 | aaaacatgacgttcaaaaaa             | ss  |
| 856 | aaaacatgacgttcgggggg             | ss  |
| 857 | ggggcatgagcttcgggggg             | sos |
| 858 | ctaggctgacgtcatcaagctagt         | o   |
| 859 | tctgacgtcatctgacgttggtgacgtct    | o   |
| 860 | ggaattagtaatagatatagaagtt        | o   |
| 861 | tttaccttttataaacataactaaaacaaa   | o   |
| 862 | gcgtttttttttgcg                  | s   |
| 863 | atatctaatacaaacattaacaaa         | o   |
| 864 | tctatcccagggtggttcctgtag         | o   |
| 865 | btccatgacgttcctgatgct            | o   |

|     |                                 |          |
|-----|---------------------------------|----------|
| 866 | btccatgagcttcctgatgct           | o        |
| 867 | ttttttttttttttf                 | o        |
| 868 | ttttttttttttttf                 | so       |
| 869 | ctagcttgatgagctcagccgctag       | o        |
| 870 | ttcagttgtcttgctgcttagctaa       | o        |
| 871 | tccatgagcttcctgagct             | s        |
| 872 | ctagcggctgacgtcatcaatctag       | o        |
| 873 | tgctagctgtgcctgtacct            | s        |
| 874 | atgctaaaggacgtcacattgca         | o        |
| 875 | tgcaatgtgacgtccttttagcat        | o        |
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| 877 | cataggatctcgagctcggaaagtcccctac | o        |
| 878 | ctgtcaggaactgcaggtaagg          | o        |
| 879 | cataacataggaatatctactcctcgc     | o        |
| 880 | ctccagctccaagaaaggacg           | o        |
| 881 | gaagtttctggttaagtcttcg          | o        |
| 882 | tgctgcttttgtgcttttgtgctt        | s        |
| 883 | tcgtcgttttgtggttttgtggtt        | s        |
| 884 | tcgtcgttttgtcgttttgtcgtt        | s        |
| 885 | tcctgacgttcggcgcgcgccc          | s        |
| 886 | tgctgcttttgtgcttttgtgctt        | s        |
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| 888 | tcgtcgttttgcgttttgcagctt        | s        |
| 889 | tcgtcgttttgcgtgcttttgcgtt       | s        |
| 890 | tcgcgtgcgttttgcgttttgcagctt     | s        |
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| 894 | tcctggcgttgaagt                 | s        |
| 895 | tcctggcgttgaagt                 | s        |
| 896 | tcctgacgtggaagt                 | s        |
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| 900 | gcggcgttcggcgcgcgccc            | s        |
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| 904 | tcgtcgtctgtctcgc                | s        |
| 905 | tgtgggggttttgggttttgg           | s        |
| 906 | aggggaggggaggggagggg            | s        |
| 907 | tgtgtgtgtgtgtgtgtgtgt           | chimeric |
| 908 | ctctctctctctctctctctct          | s        |
| 909 | ggggtcgacgtcgagggggg            | s        |
| 910 | atatatatatatatatatat            | s        |
| 911 | tttttttttttttttttttttttt        | s        |
| 912 | tttttttttttttttttttttt          | s        |
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| 914 | gctagaggggagggg                 |          |
| 915 | gctagatgttagggg                 |          |
| 916 | gcatgagggggagct                 |          |
| 917 | atggaaggtccagggggctc            |          |

|     |                          |     |
|-----|--------------------------|-----|
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| 919 | atggaaggtccaaggggctc     |     |
| 920 | gagaaggggggaccttggat     |     |
| 921 | gagaaggggggaccttccat     |     |
| 922 | gagaagggggccagcactgat    |     |
| 923 | tccatgtggggcctgatgct     |     |
| 924 | tccatgaggggcctgatgct     |     |
| 925 | tccatgtggggcctgatgat     |     |
| 926 | atggactctccgggggttctc    |     |
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| 928 | atggactctggaggggttctc    |     |
| 929 | atggaggctccatggggctc     |     |
| 930 | atggactctgggggggttctc    |     |
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| 936 | tccatgggggttctgatgct     |     |
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| 938 | gctagaggagtggt           |     |
| 939 | tttttttttttttttttt       | s   |
| 940 | gmgtcaacgttgagggmgtg     | s   |
| 941 | ggggagttcggttgagggggg    | s   |
| 942 | tcgtcggtttcccccccccc     | s   |
| 943 | ttgggggggttttttttttttt   | s   |
| 944 | tttaaattttaaaatttaaaata  | s   |
| 945 | ttgggttttttggttttttttg   | s   |
| 946 | ttcccttttccccttttccctc   | s   |
| 947 | ggggtcacgatgagggggg s    | sos |
| 948 | tccatgacgttcctgacgtt     |     |
| 949 | tccatgacgttcctgacgtt     |     |
| 950 | tccatgacgttcctgacgtt     |     |
| 951 | tccatgacgttcctgacgtt     |     |
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| 954 | tccatgacgttcctgacgtt     |     |
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| 957 | tccatgacgttcctgacgtt     |     |
| 958 | tccatgacgttcctgacgtt     |     |
| 959 | gggggacgatcgtcggggg      | sos |
| 960 | gggggtcgtagcagggggg      | sos |
| 961 | tttttttttttttttttttt     | po  |
| 962 | aaaaaaaaaaaaaaaaaaaaa    | po  |
| 963 | cccccccccccccccccccc     | po  |
| 964 | tcgtcggtttgtcggtttgtcggt |     |
| 965 | tcgtcggtttgtcggtttgtcggt |     |
| 966 | tcgtcggtttgtcggtttgtcggt |     |
| 967 | tcgtcggtttgtcggtttgtcggt |     |
| 968 | ggggtcacgttgagggggg      |     |
| 969 | ggggtcacgttgagggggg      |     |

|      |                             |     |
|------|-----------------------------|-----|
| 970  | ggggtcaagcttgagggggg        |     |
| 971  | tgtgtcttccccccccccc         |     |
| 972  | ggggacgtcgacgtgggggg        | sos |
| 973  | ggggtcgtcgacgagggggg        | sos |
| 974  | ggggtcgacgtacgtcgagggggg    | sos |
| 975  | ggggaccggtaccggtgggggg      | sos |
| 976  | gggtcgacgtcgagggggg         | sos |
| 977  | ggggtcgacgtcgaggggg         | sos |
| 978  | ggggaacgttaacgttgggggg      | sos |
| 979  | ggggtcacggtgagggggg         | sos |
| 980  | ggggtcgttcgaacgagggggg      | sos |
| 981  | ggggacgttcgaacgtgggggg      | sos |
| 982  | tcaactttga                  | s   |
| 983  | tcaagcttga                  | s   |
| 984  | tcacgatcgtga                | s   |
| 985  | tcagcatgctga                | s   |
| 986  | gggggagcatgctggggggg        | sos |
| 987  | gggggggggggggggggggg        | sos |
| 988  | gggggacgatatcgtcgggggg      | sos |
| 989  | gggggacgacgtcgtcgggggg      | sos |
| 990  | gggggacgagctcgtcgggggg      | sos |
| 991  | gggggacgtacgtcgggggg        | sos |
| 992  | tcaacgtt                    |     |
| 993  | tccataccggtcctgatgct        |     |
| 994  | tccataccggtcctaccggt        | s   |
| 995  | gggggacgatcgttgggggg        | sos |
| 996  | ggggaacgatcgtcgggggg        | sos |
| 997  | ggg ggg acg atc gtc ggg ggg | sos |
| 998  | ggg gga cga tcg tcg ggg ggg | sos |
| 999  | aaa gac gtt aaa             | po  |
| 1000 | aaagagcttaaa                | po  |
| 1001 | aaagazgttaaa                | po  |
| 1002 | aaattcggaaaa                | po  |
| 1003 | gggggtcatcgatgagggggg       | sos |
| 1004 | gggggtcaacgttgagggggg       | sos |
| 1005 | atgtagcttaataacaaagc        | po  |
| 1006 | ggatcccttgagttacttct        | po  |
| 1007 | ccattccacttctgattacc        | po  |
| 1008 | tatgtattatcatgtagata        | po  |
| 1009 | agcctacgtattcaccctcc        | po  |
| 1010 | ttcctgcaactactattgta        | po  |
| 1011 | atagaaggccctacaccagt        | po  |
| 1012 | ttacaccggtctatggaggt        | po  |
| 1013 | ctaaccagatcaagtctagg        | po  |
| 1014 | cctagacttgatctggttag        | po  |
| 1015 | tataagcctcgtccgacatg        | po  |
| 1016 | catgtcggacgaggcttata        | po  |
| 1017 | tgggtggtggggagtaagctc       | po  |
| 1018 | gagctactccccaccacca         | po  |
| 1019 | gccttcgatcttcgttggga        | po  |

|      |                                     |     |
|------|-------------------------------------|-----|
| 1020 | tggacttctctttgccgtct                | po  |
| 1021 | atgctgtagcccagcgataa                | po  |
| 1022 | accgaatcagcggaaagtga                | po  |
| 1023 | tccatgacgttcctgacgtt                |     |
| 1024 | ggagaaacccatgagctcatctgg            |     |
| 1025 | accacagaccagcaggcaga                |     |
| 1026 | gagcgtgaactgcgcgaaga                |     |
| 1027 | tcggtacccttgacgcggtt                |     |
| 1028 | ctggagccctagccaaggat                |     |
| 1029 | gcgactccatcaccagcgat                |     |
| 1030 | cctgaagtaagaaccagatgt               |     |
| 1031 | ctgtgttatctgacatacacc               |     |
| 1032 | aattagccttaggtgattggg               |     |
| 1033 | acatctggttcttacttcagg               |     |
| 1034 | ataagtcataTTTTGGGAactac             |     |
| 1035 | cccaatcacctaaggctaatt               |     |
| 1036 | ggggtcgtcgacgagggggg                | sos |
| 1037 | ggggtcgttcgaacgagggggg              | sos |
| 1038 | ggggacgttcgaacgtgggggg              | sos |
| 1039 | tcctggcgqggaagt                     | s   |
| 1040 | ggggaacgacgtcgttgggggg              | sos |
| 1041 | ggggaacgtacgtcgggggg                | sos |
| 1042 | ggggaacgtacgtacgttgggggg            | sos |
| 1043 | ggggtcaccggtgagggggg                | sos |
| 1044 | ggggtcgacgtacgtcgagggggg            | sos |
| 1045 | ggggaccggtaccggtgggggg              | sos |
| 1046 | gggtcgacgtcgagggggg                 | sos |
| 1047 | ggggtcgacgtcgagggg                  | sos |
| 1048 | ggggaacgttaacgttgggggg              | sos |
| 1049 | ggggacgtcgacgtggggg                 | sos |
| 1050 | gcactcttcgaagctacagccggcagcctctgat  |     |
| 1051 | cggctcttccatgaggtctttgctaattcttgg   |     |
| 1052 | cggctcttccatgaaagtctttggacgatgtgagc |     |
| 1053 | tcctgcaggttaagt                     | s   |
| 1054 | gggggtcgttcgttgggggg                | sos |
| 1055 | gggggatgattgttgggggg                | sos |
| 1056 | gggggazgatzgttgggggg                | sos |
| 1057 | gggggagctagcttgggggg                | sos |
| 1058 | ggttcttttggtccttgtct                | s   |
| 1059 | ggttcttttggtcctcgtct                | s   |
| 1060 | ggttcttttggtccttatct                | s   |
| 1061 | ggttcttggttcccttgtct                | s   |
| 1062 | tggtcttttggtccttgtct                | s   |
| 1063 | ggttcaaatggtccttgtct                | s   |
| 1064 | gggtcttttgggccttgtct                | s   |
| 1065 | tccaggacttctctcaggTTTTT             | s   |
| 1066 | tccaaaacttctctcaaat                 | s   |
| 1067 | tactacttttatacttttatactt            | s   |
| 1068 | tgtgtgtgtgtgtgtgtgtgtg              | s   |
| 1069 | ttgttgttgttgttgttgttgtg             | s   |



|      |                              |     |
|------|------------------------------|-----|
| 1070 | ggctccggggaggggaatttttgtctat | s   |
| 1071 | gggacgatcgtcggggggg          | sos |
| 1072 | gggtcgtcgacgagggggg          | sos |
| 1073 | ggtcgtcgacgagggggg           | sos |
| 1074 | gggtcgtcgtcgtggggggg         | sos |
| 1075 | ggggacgatcgtcggggggg         | sos |
| 1076 | ggggacgtcgtcgtggggggg        | sos |
| 1077 | ggggtcgacgtcgacgtcgagggggg   | sos |
| 1078 | ggggaaccgcggttggggggg        | sos |
| 1079 | ggggacgacgtcgtggggggg        | sos |
| 1080 | tcgtcgtcgtcgtcgtggggggg      | sos |
| 1081 | tcttgccggggaagt              | s   |
| 1082 | tctgcaggggaagt               | s   |
| 1083 | tctgaagggaagt                | s   |
| 1084 | tctggcgggcaagt               | s   |
| 1085 | tctggcgggtaagt               | s   |
| 1086 | tctggcgggaaagt               | s   |
| 1087 | tccgggcggggaagt              | s   |
| 1088 | tcggggcggggaagt              | s   |
| 1089 | tccgggcggggaagt              | s   |
| 1090 | gggggacgttggggg              | s   |
| 1091 | ggggtttttttttgggggg          | sos |
| 1092 | ggggcccccccccgggggg          | sos |
| 1093 | ggggttgttgttgggggg           | sos |

In some embodiments, the immunostimulatory nucleic acid is a CpG nucleic acid. CpG sequences, while relatively rare in human DNA are commonly found in the DNA of infectious organisms such as bacteria. The human immune system has apparently evolved to recognize CpG sequences as an early warning sign of infection and to initiate an immediate and powerful immune response against invading pathogens without causing adverse reactions frequently seen with other immune stimulatory agents. Thus CpG containing nucleic acids, relying on this innate immune defense mechanism can utilize a unique and natural pathway for immune therapy. The effects of CpG nucleic acids on immune modulation have been described extensively in published patent applications, such as PCT US95/01570), PCT/US97/19791, PCT/US98/03678; PCT/US98/10408; PCT/US98/04703; PCT/US99/07335; and PCT/US99/09863. The entire contents of each of these patent applications is hereby incorporated by reference.

A CpG nucleic acid is a nucleic acid which includes at least one unmethylated CpG dinucleotide. A nucleic acid containing at least one unmethylated CpG dinucleotide is a nucleic acid molecule which contains an unmethylated cytosine in a cytosine-guanine dinucleotide sequence (i.e. "CpG DNA" or DNA containing a 5' cytosine followed by 3' guanosine and linked by a phosphate bond) and activates the immune system. The CpG

nucleic acids can be double-stranded or single-stranded. Generally, double-stranded molecules are more stable *in vivo*, while single-stranded molecules have increased immune activity. Thus in some aspects of the invention it is preferred that the nucleic acid be single stranded and in other aspects it is preferred that the nucleic acid be double stranded. The terms CpG nucleic acid or CpG oligonucleotide as used herein refer to an immunostimulatory CpG nucleic acid or a nucleic acid unless otherwise indicated. The entire immunostimulatory nucleic acid can be unmethylated or portions may be unmethylated but at least the C of the 5' CG 3' must be unmethylated.

In one preferred embodiment the invention provides an immunostimulatory nucleic acid which is a CpG nucleic acid represented by at least the formula:



wherein  $X_1$ ,  $X_2$ ,  $X_3$ , and  $X_4$  are nucleotides. In one embodiment  $X_2$  is adenine, guanine, cytosine, or thymine. In another embodiment  $X_3$  is cytosine, guanine, adenine, or thymine. In other embodiments  $X_2$  is adenine, guanine, or thymine and  $X_3$  is cytosine, adenine, or thymine.

In another embodiment the immunostimulatory nucleic acid is an isolated CpG nucleic acid represented by at least the formula:



wherein  $X_1$ ,  $X_2$ ,  $X_3$ , and  $X_4$  are nucleotides and N is any nucleotide and  $N_1$  and  $N_2$  are nucleic acid sequences composed of from about 0-25 N's each. In one embodiment  $X_1X_2$  are nucleotides selected from the group consisting of: GpT, GpG, GpA, ApA, ApT, ApG, CpT, CpA, CpG, TpA, TpT, and TpG; and  $X_3X_4$  are nucleotides selected from the group consisting of: TpT, ApT, TpG, ApG, CpG, TpC, ApC, CpC, TpA, ApA, and CpA. Preferably  $X_1X_2$  are GpA or GpT and  $X_3X_4$  are TpT. In other embodiments  $X_1$  or  $X_2$  or both are purines and  $X_3$  or  $X_4$  or both are pyrimidines or  $X_1X_2$  are GpA and  $X_3$  or  $X_4$  or both are pyrimidines. In another preferred embodiment  $X_1X_2$  are nucleotides selected from the group consisting of: TpA, ApA, ApC, ApG, and GpG. In yet another embodiment  $X_3X_4$  are nucleotides selected from the group consisting of: TpT, TpA, TpG, ApA, ApG, ApC, and CpA.  $X_1X_2$  in another embodiment are nucleotides selected from the group consisting of: TpT, TpG, ApT, GpC, CpC, CpT, TpC, GpT and CpG.

In another preferred embodiment the immunostimulatory nucleic acid has the sequence 5'TCN<sub>1</sub>TX<sub>1</sub>X<sub>2</sub>CGX<sub>3</sub>X<sub>4</sub>3'. The immunostimulatory nucleic acids of the invention in

some embodiments include  $X_1X_2$  selected from the group consisting of GpT, GpG, GpA and ApA and  $X_3X_4$  is selected from the group consisting of TpT, CpT and TpC.

In other embodiments, the CpG oligonucleotide has a sequence selected from the group consisting of SEQ ID NO: 1, 3, 4, 14-16, 18-24, 28, 29, 33-46, 49, 50, 52-56, 58, 64-  
5 67, 69, 71, 72, 76-87, 90, 91, 93, 94, 96, 98, 102-124, 126-128, 131-133, 136-141, 146-150, 152-153, 155-171, 173-178, 180-186, 188-198, 201, 203-214, 216-220, 223, 224, 227-240, 242-256, 258, 260-265, 270-273, 275, 277-281, 286-287, 292, 295-296, 300, 302, 305-307, 309-312, 314-317, 320-327, 329, 335, 337-341, 343-352, 354, 357, 361-365, 367-369, 373-376, 378-385, 388-392, 394, 395, 399, 401-404, 406-426, 429-433, 434-437, 439, 441-443,  
10 445, 447, 448, 450, 453-456, 460-464, 466-469, 472-475, 477, 478, 480, 483-485, 488, 489, 492, 493, 495-502, 504-505, 507-509, 511, 513-529, 532-541, 543-555, 564-566, 568-576, 578, 580, 599, 601-605, 607-611, 613-615, 617, 619-622, 625-646, 648-650, 653-664, 666-697, 699-706, 708, 709, 711-716, 718-732, 736, 737, 739-744, 746, 747, 749-761, 763, 766-767, 769, 772-779, 781-783, 785-786, 7900792, 798-799, 804-808, 810, 815, 817, 818, 820-  
15 832, 835-846, 849-850, 855-859, 862, 865, 872, 874-877, 879-881, 883-885, 888-904, and 909-913.

For facilitating uptake into cells, the immunostimulatory nucleic acids are preferably in the range of 6 to 100 bases in length. However, nucleic acids of any size greater than 6 nucleotides (even many kb long) are capable of inducing an immune response according to  
20 the invention if sufficient immunostimulatory motifs are present. Preferably the immunostimulatory nucleic acid is in the range of between 8 and 100 and in some embodiments between 8 and 50 or 8 and 30 nucleotides in size.

“Palindromic sequence” shall mean an inverted repeat (i.e. a sequence such as ABCDEE'D'C'B'A' in which A and A' are bases capable of forming the usual Watson-Crick  
25 base pairs. *In vivo*, such sequences may form double-stranded structures. In one embodiment the CpG nucleic acid contains a palindromic sequence. A palindromic sequence used in this context refers to a palindrome in which the CpG is part of the palindrome, and preferably is the center of the palindrome. In another embodiment the CpG nucleic acid is free of a palindrome. An immunostimulatory nucleic acid that is free of a palindrome is one in which  
30 the CpG dinucleotide is not part of a palindrome. Such an oligonucleotide may include a palindrome in which the CpG is not the center of the palindrome.

The CpG nucleic acid sequences of the invention are those broadly described above as well as disclosed in PCT Published Patent Applications PCT/US95/01570 and

PCT/US97/19791 claiming priority to U.S. Serial Nos. 08/386,063 and 08/960,774, filed on February 7, 1995 and October 30, 1997 respectively.

The immunostimulatory nucleic acids of the invention also include nucleic acids having T-rich motifs. It was recently discovered by Dr. Arthur Krieg that T-rich nucleic acids were immunostimulatory. It was presented by Dr. Krieg at the International Workshop on "Immunobiology of Bacterial CpG-DNA" held in Upper Bavaria on September 26 - 29, 1999 that poly-T nucleic acids of 24 bases in length are immunostimulatory, whereas the same length poly-C oligonucleotide is non-stimulatory. These concepts are also described and claimed in US Provisional Patent Application No. 60/156,113 filed on September 25, 1999, which is hereby incorporated by reference.

Poly-G containing nucleic acids are also immunostimulatory. PCT published patent application number WO 00/14217, which claims priority to German Patent Application No. 98 11 6652.3, filed on September 3, 1998 describes poly-G-containing oligonucleotides and their uses. A variety of other references, including Pisetsky and Reich, 1993 *Mol. Biol. Reports*, 18:217-221; Krieger and Herz, 1994, *Ann. Rev. Biochem.*, 63:601-637; Macaya et al., 1993, *PNAS*, 90:3745-3749; Wyatt et al., 1994, *PNAS*, 91:1356-1360; Rando and Hogan, 1998, In *Applied Antisense Oligonucleotide Technology*, ed. Krieg and Stein, p. 335-352; and Kimura et al., 1994, *J. Biochem.* 116, 991-994 also describe the immunostimulatory properties of poly-G nucleic acids. Poly-G-containing nucleotides are useful for treating and preventing bacterial and viral infections.

In some aspects of the invention the poly-G containing nucleic acids are administered alone for the treatment of asthma and allergy. It was previously suggested in the prior art that poly-G rich oligonucleotides inhibit the production of IFN- $\delta$  by compounds such as CpG oligonucleotides, concanavalin A, bacterial DNA, or the combination of PMA and the calcium ionophore A 23187 (Halperin and Pisetsky, 1995, *Immunopharmacol.*, 29:47-52, as well as block the downstream effects of IFN- $\delta$ . For instance, Ramanathan et al., 1994, *Transplantation*, 57:612-615, has shown that a poly-G oligonucleotide inhibits the binding of IFN- $\delta$  to its receptor, which prevents the normal enhancement of MHC Class 1 and ICAM-1 in response to IFN- $\delta$ . Poly-G oligonucleotides were also found to be able to inhibit the secretion of IFN- $\delta$  from lymphocytes (Halperin and Pisetsky, 1995, *Immunopharmacol.*, 29:47-52). It was surprisingly, discovered according to the invention that when poly-G nucleic acids are administered *in vivo*, they are useful for treating or preventing allergy or

asthma. Thus, in this aspect of the invention, poly-G nucleic acids are administered alone or optionally with other asthma/allergy medicaments for the treatment of allergy and/or asthma.

Poly-G nucleic acids preferably are nucleic acids having the following formulas:



5 wherein  $X_1$ ,  $X_2$ ,  $X_3$ , and  $X_4$  are nucleotides. In preferred embodiments at least one of  $X_3$  and  $X_4$  are a G. In other embodiments both of  $X_3$  and  $X_4$  are a G. In yet other embodiments the preferred formula is  $5' GGGNGGG 3'$ , or  $5' GGGNGGGNGGG 3'$  wherein N represents between 0 and 20 nucleotides. In other embodiments the poly-G nucleic acid is free of unmethylated CG dinucleotides, while in other embodiments the poly-G nucleic acid includes  
10 at least one unmethylated CG dinucleotide.

The poly G nucleic acid in some embodiments is selected from the group consisting of SEQ ID NO: 5, 6, 73, 215, 267-269, 276, 282, 288, 297-299, 355, 359, 386, 387, 444, 476, 531, 557-559, 733, 768, 795, 796, 914-925, 928-931, 933-936, and 938. In other  
15 embodiments, the poly G nucleic acid includes a sequence selected from the group consisting of SEQ ID NO: 67, 80-82, 141, 147, 148, 173, 178, 183, 185, 214, 224, 264, 265, 315, 329, 434, 435, 475, 519, 521-524, 526, 527, 535, 554, 565, 609, 628, 660, 661, 662, 725, 767, 825, 856, 857, 876, 892, 909, 926, 927, 932, and 937. In some embodiments, the entire backbone of the poly-G nucleic acid is phosphorothioate.

In related embodiments, the invention also contemplates the use of  
20 immunostimulatory nucleic acids that comprise one and preferably two poly-G motifs, even more preferably flanking a palindrome. Such immunostimulatory nucleic acids preferably have a chimeric backbone (i.e., their backbone is comprised of both phosphodiester and phosphorothioate linkages). Even more preferably, the phosphorothioate linkages in these latter immunostimulatory nucleic acids are located at the 5' and 3' ends of the nucleic acid.  
25 Examples of suitable palindromes include, but are not limited to AACGTT; AAGCTT; AGCGCT; TCGA; TTCGAA; ACGT; GACGTC ; and CACGTG.

Nucleic acids having modified backbones, such as phosphorothioate backbones, fall within the class of immunostimulatory nucleic acids. U.S. Patents Nos. 5,723,335 and 5,663,153 issued to Hutcherson, et al. and related PCT publication WO95/26204 describe  
30 immune stimulation using phosphorothioate oligonucleotide analogues. These patents describe the ability of the phosphorothioate backbone to stimulate an immune response in a non-sequence specific manner.

The backbone characteristics of the nucleic acids listed in Table 1 are also shown. Some of the designations in the Table are as follows: o or po = phosphodiester, s = phosphorothioate, sos = chimeric.

In the case when the immunostimulatory nucleic acid is administered in conjunction with a nucleic acid vector, it is preferred that the backbone of the immunostimulatory nucleic acid be a chimeric combination of phosphodiester and phosphorothioate (or other phosphate modification). The cell may have a problem taking up a plasmid vector in the presence of completely phosphorothioate oligonucleotide. Thus when both a vector and an oligonucleotide are delivered to a subject, it is preferred that the oligonucleotide have a chimeric backbone or have a phosphorothioate backbone but that the plasmid is associated with a vehicle that delivers it directly into the cell, thus avoiding the need for cellular uptake. Such vehicles are known in the art and include, for example, liposomes and gene guns.

For use in the instant invention, the immunostimulatory nucleic acids can be synthesized *de novo* using any of a number of procedures well known in the art. Such compounds are referred to as "synthetic nucleic acids." For example, the b-cyanoethyl phosphoramidite method (Beaucage, S.L., and Caruthers, M.H., *Tet. Let.* 22:1859, 1981); nucleoside H-phosphonate method (Garegg *et al.*, *Tet. Let.* 27:4051-4054, 1986; Froehler *et al.*, *Nucl. Acid. Res.* 14:5399-5407, 1986, ; Garegg *et al.*, *Tet. Let.* 27:4055-4058, 1986, Gaffney *et al.*, *Tet. Let.* 29:2619-2622, 1988). These chemistries can be performed by a variety of automated oligonucleotide synthesizers available in the market. These nucleic acids are referred to as synthetic nucleic acids. Alternatively, immunostimulatory nucleic acids can be produced on a large scale in plasmids, (see Sambrook, T., *et al.*, "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor laboratory Press, New York, 1989) and separated into smaller pieces or administered whole. Nucleic acids can be prepared from existing nucleic acid sequences (*e.g.*, genomic or cDNA) using known techniques, such as those employing restriction enzymes, exonucleases or endonucleases. Nucleic acids prepared in this manner are referred to as isolated nucleic acids. The term "immunostimulatory nucleic acid" encompasses both synthetic and isolated immunostimulatory nucleic acids.

For use *in vivo*, nucleic acids are preferably relatively resistant to degradation (*e.g.*, are stabilized). A "stabilized nucleic acid molecule" shall mean a nucleic acid molecule that is relatively resistant to *in vivo* degradation (*e.g.* via an exo- or endo-nuclease). Stabilization can be a function of length or secondary structure. Immunostimulatory nucleic acids that are tens to hundreds of kbs long are relatively resistant to *in vivo* degradation. For shorter

immunostimulatory nucleic acids, secondary structure can stabilize and increase their effect. For example, if the 3' end of a nucleic acid has self-complementarity to an upstream region, so that it can fold back and form a sort of stem loop structure, then the nucleic acid becomes stabilized and therefore exhibits more activity.

5 Alternatively, nucleic acid stabilization can be accomplished via backbone modifications. Preferred stabilized nucleic acids of the instant invention have a modified backbone. It has been demonstrated that modification of the nucleic acid backbone provides enhanced activity of the immunostimulatory nucleic acids when administered *in vivo*. One type of modified backbone is a phosphate backbone modification. Immunostimulatory  
10 nucleic acids, including at least two phosphorothioate linkages at the 5' end of the oligonucleotide and multiple phosphorothioate linkages at the 3' end, preferably 5, can in some circumstances provide maximal activity and protect the nucleic acid from degradation by intracellular exo- and endo-nucleases. Other phosphate modified nucleic acids include phosphodiester modified nucleic acids, combinations of phosphodiester and phosphorothioate  
15 nucleic acids, methylphosphonate, methylphosphorothioate, phosphorodithioate, and combinations thereof. Each of these combinations in CpG nucleic acids and their particular effects on immune cells is discussed in more detail in PCT Published Patent Applications PCT/US95/01570 and PCT/US97/19791, the entire contents of which are hereby incorporated by reference. Although Applicants are not bound by the theory, it is believed that these  
20 phosphate modified nucleic acids may show more stimulatory activity due to enhanced nuclease resistance, increased cellular uptake, increased protein binding, and/or altered intracellular localization.

Modified backbones such as phosphorothioates may be synthesized using automated techniques employing either phosphoramidate or H-phosphonate chemistries. Aryl- and  
25 alkyl-phosphonates can be made, *e.g.*, as described in U.S. Patent No. 4,469,863; and alkylphosphotriesters (in which the charged oxygen moiety is alkylated as described in U.S. Patent No. 5,023,243 and European Patent No. 092,574) can be prepared by automated solid phase synthesis using commercially available reagents. Methods for making other DNA backbone modifications and substitutions have been described (Uhlmann, E. and Peyman, A.,  
30 *Chem. Rev.* 90:544, 1990; Goodchild, J., *Bioconjugate Chem.* 1:165, 1990).

Both phosphorothioate and phosphodiester nucleic acids containing immunostimulatory motifs are active in immune cells. However, based on the concentration needed to induce immunostimulatory nucleic acid specific effects, the nuclease resistant

phosphorothioate backbone immunostimulatory nucleic acids are more potent (2 µg/ml for the phosphorothioate vs. a total of 90 µg/ml for phosphodiester).

Another type of modified backbone, useful according to the invention, is a peptide nucleic acid. The backbone is composed of aminoethylglycine and supports bases which provide the DNA-character. The backbone does not include any phosphate and thus may optionally have no net charge. The lack of charge allows for stronger DNA-DNA binding because the charge repulsion between the two strands does not exist. Additionally, because the backbone has an extra methylene group, the oligonucleotides are enzyme/protease resistant. Peptide nucleic acids can be purchased from various commercial sources, e.g., Perkin Elmer, C. A. or synthesized de novo.

Another class of backbone modifications include 2'-O-methylribonucleosides (2'-Ome). These types of substitutions are described extensively in the prior art and in particular with respect to their immunostimulating properties in Zhao et al., *Bioorganic and Medicinal Chemistry Letters*, 1999, 9:24:3453. Zhao et al. describes methods of preparing 2'-Ome modifications to nucleic acids.

The nucleic acid molecules of the invention may include naturally-occurring or synthetic purine or pyrimidine heterocyclic bases as well as modified backbones. Purine or pyrimidine heterocyclic bases include, but are not limited to, adenine, guanine, cytosine, thymidine, uracil, and inosine. Other representative heterocyclic bases are disclosed in U.S. Patent No. 3,687,808, issued to Merigan, et al. The term purine or pyrimidine or bases are used herein to refer to both naturally-occurring or synthetic purines, pyrimidines or bases.

Other stabilized nucleic acids include: nonionic DNA analogs, such as alkyl- and aryl-phosphates (in which the charged phosphonate oxygen is replaced by an alkyl or aryl group), phosphodiester and alkylphosphotriesters, in which the charged oxygen moiety is alkylated. Nucleic acids which contain diol, such as tetraethyleneglycol or hexaethyleneglycol, at either or both termini have also been shown to be substantially resistant to nuclease degradation.

The immunostimulatory nucleic acids having backbone modifications useful according to the invention in some embodiments are S- or R-chiral immunostimulatory nucleic acids. An "S chiral immunostimulatory nucleic acid" as used herein is an immunostimulatory nucleic acid wherein at least two nucleotides have a backbone modification forming a chiral center and wherein a plurality of the chiral centers have S chirality. An "R chiral immunostimulatory nucleic acid" as used herein is an immunostimulatory nucleic acid wherein at least two nucleotides have a backbone



modification forming a chiral center and wherein a plurality of the chiral centers have R  
chirality. The backbone modification may be any type of modification that forms a chiral  
center. The modifications include but are not limited to phosphorothioate,  
methylphosphonate, methylphosphorothioate, phosphorodithioate, 2'-Ome and combinations  
thereof.

The chiral immunostimulatory nucleic acids must have at least two nucleotides within  
the nucleic acid that have a backbone modification. All or less than all of the nucleotides in  
the nucleic acid, however, may have a modified backbone. Of the nucleotides having a  
modified backbone (referred to as chiral centers), a plurality have a single chirality, S or R. A  
"plurality" as used herein within the context of modified backbones refers to an amount  
greater than 50%. Thus, less than all of the chiral centers may have S or R chirality as long as  
a plurality of the chiral centers have S or R chirality. In some embodiments at least 55%,  
60% , 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the chiral centers have S or R  
chirality. In other embodiments at least 55%, 60% , 65%, 70%, 75%, 80%, 85%, 90%, 95%,  
or 100% of the nucleotides have backbone modifications.

The S- and R- chiral immunostimulatory nucleic acids may be prepared by any  
method known in the art for producing chirally pure oligonucleotides. The Stec et al  
reference teaches methods for producing stereopure phosphorothioate oligodeoxynucleotides  
using an oxathiaphospholane. (Stec, W.J., et al., 1995, *J. Am. Chem. Soc.*, 117:12019). Other  
methods for making chirally pure oligonucleotides have been described by companies such as  
ISIS Pharmaceuticals. US Patents have also described these methods. For instance U.S  
Patent Nos. 5883237; 5837856; 5599797; 5512668; 5856465; 5359052; 5506212; 5521302;  
and 5212295, each of which is hereby incorporated by reference in its entirety, disclose  
methods for generating stereopure oligonucleotides.

The immunostimulatory nucleic acids are useful for treating or preventing allergy or  
asthma in a subject. A "subject" shall mean a human or vertebrate mammal including but not  
limited to a dog, cat, horse, cow, pig, sheep, goat, or primate, e.g., monkey.

The immunostimulatory nucleic acids are useful in some aspects of the invention as a  
prophylactic for the treatment of a subject at risk of developing an allergy or asthma where  
the exposure of the subject to an allergen or predisposition to asthma is known or suspected.  
A "subject at risk" of developing allergy or asthma as used herein is a subject who has any  
risk of exposure to an allergen or a risk of developing asthma, i.e. someone who has suffered  
from an asthmatic attack previously or has a predisposition to asthmatic attacks. For instance,

a subject at risk may be a subject who is planning to travel to an area where a particular type of allergen or asthmatic initiator is found or it may even be any subject living in an area where an allergen has been identified. If the subject develops allergic responses to a particular antigen and the subject may be exposed to the antigen, i.e., during pollen season, then that  
5 subject is at risk of exposure to the antigen. A subject at risk of developing an allergy or asthma includes those subjects that have been identified as having an allergy or asthma but that don't have the active disease during the treatment of the invention as well as subjects that are considered to be at risk of developing these diseases because of genetic or environmental factors.

10 In addition to the use of the immunostimulatory nucleic acid and the asthma/allergy medicament for prophylactic treatment, the invention also encompasses the use of the combination of drugs for the treatment of a subject having an allergy or asthma. A "subject having an allergy" is a subject that has an allergic reaction in response to an allergen. An "allergy" refers to acquired hypersensitivity to a substance (allergen).

15 The allergic reaction in man and animals has been extensively studied and the basic immune mechanisms involved are well known. Allergic conditions or diseases in humans include but are not limited to eczema, allergic rhinitis or coryza, hay fever, conjunctivitis, bronchial or allergic asthma, urticaria (hives) and food allergies; atopic dermatitis; anaphylaxis; drug allergy; angioedema; and allergic conjunctivitis. Allergic diseases in dogs  
20 include but are not limited to seasonal dermatitis; perennial dermatitis; rhinitis; conjunctivitis; allergic asthma; and drug reactions. Allergic diseases in cats include but are not limited to dermatitis and respiratory disorders; and food allergens. Allergic diseases in horses include but are not limited to respiratory disorders such as "heaves" and dermatitis. Allergic diseases in non-human primates include but are not limited to allergic asthma and allergic dermatitis.

25 The generic name for molecules that cause an allergic reaction is allergen. There are numerous species of allergens. The allergic reaction occurs when tissue-sensitizing immunoglobulin of the IgE type reacts with foreign allergen. The IgE antibody is bound to mast cells and/or basophils, and these specialized cells release chemical mediators (vasoactive amines) of the allergic reaction when stimulated to do so by allergens bridging the ends of the  
30 antibody molecule. Histamine, platelet activating factor, arachidonic acid metabolites, and serotonin are among the best known mediators of allergic reactions in man. Histamine and the other vasoactive amines are normally stored in mast cells and basophil leukocytes. The mast cells are dispersed throughout animal tissue and the basophils circulate within the

vascular system. These cells manufacture and store histamine within the cell unless the specialized sequence of events involving IgE binding occurs to trigger its release.

The symptoms of the allergic reaction vary, depending on the location within the body where the IgE reacts with the antigen. If the reaction occurs along the respiratory epithelium the symptoms are sneezing, coughing and asthmatic reactions. If the interaction occurs in the digestive tract, as in the case of food allergies, abdominal pain and diarrhea are common. Systematic reactions, for example following a bee sting, can be severe and often life threatening.

Delayed type hypersensitivity, also known as type IV allergy reaction is an allergic reaction characterized by a delay period of at least 12 hours from invasion of the antigen into the allergic subject until appearance of the inflammatory or immune reaction. The T lymphocytes (sensitized T lymphocytes) of individuals in an allergic condition react with the antigen, triggering the T lymphocytes to release lymphokines (macrophage migration inhibitory factor (MIF), macrophage activating factor (MAF), mitogenic factor (MF), skin-reactive factor (SRF), chemotactic factor, neovascularization-accelerating factor, etc.), which function as inflammation mediators, and the biological activity of these lymphokines, together with the direct and indirect effects of locally appearing lymphocytes and other inflammatory immune cells, give rise to the type IV allergy reaction. Delayed allergy reactions include tuberculin type reaction, homograft rejection reaction, cell-dependent type protective reaction, contact dermatitis hypersensitivity reaction, and the like, which are known to be most strongly suppressed by steroidal agents. Consequently, steroidal agents are effective against diseases which are caused by delayed allergy reactions. Long-term use of steroidal agents at concentrations currently being used can, however, lead to the serious side-effect known as steroid dependence. The methods of the invention solve some of these problems, by providing for lower and fewer doses to be administered.

Immediate hypersensitivity (or anaphylactic response) is a form of allergic reaction which develops very quickly, i.e. within seconds or minutes of exposure of the patient to the causative allergen, and it is mediated by IgE antibodies made by B lymphocytes. In nonallergic patients, there is no IgE antibody of clinical relevance; but, in a person suffering with allergic diseases, IgE antibody mediates immediate hypersensitivity by sensitizing mast cells which are abundant in the skin, lymphoid organs, in the membranes of the eye, nose and mouth, and in the respiratory tract and intestines.

Mast cells have surface receptors for IgE, and the IgE antibodies in allergy-suffering

patients become bound to them. As discussed briefly above, when the bound IgE is subsequently contacted by the appropriate allergen, the mast cell is caused to degranulate and to release various substances called bioactive mediators, such as histamine, into the surrounding tissue. It is the biologic activity of these substances which is responsible for the clinical symptoms typical of immediate hypersensitivity; namely, contraction of smooth muscle in the airways or the intestine, the dilation of small blood vessels and the increase in their permeability to water and plasma proteins, the secretion of thick sticky mucus, and in the skin, redness, swelling and the stimulation of nerve endings that results in itching or pain.

Many allergies are caused by IgE antibody generation against harmless allergens. The cytokines that are induced by administration of immunostimulatory nucleic acids are predominantly of a class called "Th1" (examples are IL-12 and IFN- $\gamma$ ). Cytokine production by helper CD4<sup>+</sup> (and also in CD8<sup>+</sup>) T cells frequently fall into one of two phenotypes, Th1 and Th2, in both murine and human systems (Romagnani, 1991, *Immunol Today* 12: 256-257, Mosmann, 1989, *Annu Rev Immunol*, 7: 145-173). Th1 cells produce interleukin 2 (IL-2), tumor necrosis factor (TNF $\alpha$ ) and interferon gamma (IFN $\gamma$ ) and they are responsible primarily for cell-mediated immunity such as delayed type hypersensitivity. Th2 cells produce interleukins, IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 and are primarily involved in providing optimal help for humoral immune responses such as IgE and IgG4 antibody isotype switching (Mosmann, 1989, *Annu Rev Immunol*, 7: 145-173).

The types of antibodies associated with a Th1 response are generally more protective because they have high neutralization and opsonization capabilities. Th2 responses involve predominately antibodies and these have less protective effect against infection and some Th2 isotypes (e.g., IgE) are associated with allergy. Strongly polarized Th1 and Th2 responses not only play different roles in protection, they can promote different immunopathological reactions. Th1-type responses are involved organ specific autoimmunity such as experimental autoimmune uveoretinitis (Dubey et al, 1991, *Eur Cytokine Network* 2: 147-152), experimental autoimmune encephalitis (EAE) (Beraud et al, 1991, *Cell Immunol* 133: 379-389) and insulin dependent diabetes mellitus (Hahn et al, 1987, *Eur. J. Immunol.* 18: 2037-2042), in contact dermatitis (Kapsenberg et al, *Immunol Today* 12: 392-395), and in some chronic inflammatory disorders. In contrast Th2-type responses are responsible for triggering allergic atopic disorders (against common environmental allergens) such as allergic asthma (Walker et al, 1992, *Am Rev Resp Dis* 148: 109-115) and atopic dermatitis (van der Heijden et al, 1991, *J Invest Derm* 97: 389-394), are thought to exacerbate infection with tissue-

dwelling protozoa such as helminths (Finkelman et al, 1991, Immunoparasitol Today 12: A62-66) and Leishmania major (Caceres-Dittmar et al, 1993, Clin Exp Immunol 91: 500-505), are preferentially induced in certain primary immunodeficiencies such as hyper-IgE syndrome (Del Prete et al, 1989, J Clin Invest 84: 1830-1835) and Omenn's syndrome (Schandene et al, 1993, Eur J Immunol 23: 56-60), and are associated with reduced ability to suppress HIV replication (Barker et al, 1995, Proc Soc Nat Acad Sci USA 92: 11135-11139).

Thus, in general, it appears that allergic diseases are mediated by Th2 type immune responses. Based on the ability of the immunostimulatory nucleic acid to shift the immune response in a subject from a Th2 (which is associated with production of IgE antibodies and allergy and asthma) to a Th1 response (which is protective against allergic and asthmatic reactions), an effective dose for inducing an immune response of a immunostimulatory nucleic acid can be administered to a subject to treat or prevent an allergy or asthma.

Th2 cytokines, especially IL-4 and IL-5 are elevated in the airways of asthmatic subjects. These cytokines promote important aspects of the asthmatic inflammatory response, including IgE isotype switching, eosinophil chemotaxis and activation, and mast cell growth. Th1 cytokines, especially IFN-g and IL-12, can suppress the formation of Th2 clones and production of Th2 cytokines. Thus, the immunostimulatory nucleic acid has significant therapeutic utility in the treatment of allergic conditions and asthma.

An "allergen" as used herein is a molecule capable of provoking an immune response characterized by production of IgE. Thus, in the context of this invention, the term allergen means a specific type of antigen which can trigger an allergic response which is mediated by IgE antibody. The method and preparations of this invention extend to a broad class of such allergens and fragments of allergens or haptens acting as allergens. Allergens include but are not limited to Environmental Aeroallergens; plant pollens such as Ragweed/hayfever (affects 10% of pop., 25 million ppl); Weed pollen allergens; Grass pollen allergens (grasses affect 10% of pop., 25 million ppl); Johnson grass; Tree pollen allergens; Ryegrass; House dust mite allergens (affects 6% of pop., 15 million ppl); Storage mite allergens; Japanese cedar pollen/hay fever (affects 10% of pop. In Japan, 13 million ppl); Mold spore allergens; Animal allergens (cat (affects 2% of pop., 5 million ppl), dog, guinea pig, hamster, gerbil, rat, mouse); Food Allergens (e.g., Crustaceans; nuts, such as peanuts; citrus fruits); Insect Allergens (Other than mites listed above); Venoms: (Hymenoptera, yellow jacket, honey bee, wasp, hornet, fire ant); Other environmental insect allergens from cockroaches, fleas, mosquitoes, etc.; Bacteria such as streptococcal antigens; Parasites such as Ascaris antigen; Viral

Antigens; Fungal spores; Drug Allergens; Antibiotics; penicillins and related compounds; other antibiotics; Whole Proteins such as hormones (insulin), enzymes (Streptokinase); all drugs and their metabolites capable of acting as incomplete antigens or haptens; Industrial Chemicals and metabolites capable of acting as haptens and stimulating the immune system (Examples are the acid anhydrides (such as trimellitic anhydride) and the isocyanates (such as toluene diisocyanate)); Occupational Allergens such as flour (ie. Baker's asthma), castor bean, coffee bean, and industrial chemicals described above; flea allergens; and human proteins in non-human animals.

Allergens include but are not limited to cells, cell extracts, proteins, polypeptides, peptides, polysaccharides, polysaccharide conjugates, peptide and non-peptide mimics of polysaccharides and other molecules, small molecules, lipids, glycolipids, and carbohydrates. Many allergens, however, are protein or polypeptide in nature, as proteins and polypeptides are generally more antigenic than carbohydrates or fats.

Examples of specific natural, animal and plant allergens include but are not limited to proteins specific to the following genres: *Canine* (*Canis familiaris*); *Dermatophagoides* (e.g. *Dermatophagoides farinae*); *Felis* (*Felis domesticus*); *Ambrosia* (*Ambrosia artemisiifolia*); *Lolium* (e.g. *Lolium perenne* or *Lolium multiflorum*); *Cryptomeria* (*Cryptomeria japonica*); *Alternaria* (*Alternaria alternata*); *Alder*; *Alnus* (*Alnus gultinoasa*); *Betula* (*Betula verrucosa*); *Quercus* (*Quercus alba*); *Olea* (*Olea europa*); *Artemisia* (*Artemisia vulgaris*); *Plantago* (e.g. *Plantago lanceolata*); *Parietaria* (e.g. *Parietaria officinalis* or *Parietaria judaica*); *Blattella* (e.g. *Blattella germanica*); *Apis* (e.g. *Apis multiflorum*); *Cupressus* (e.g. *Cupressus sempervirens*, *Cupressus arizonica* and *Cupressus macrocarpa*); *Juniperus* (e.g. *Juniperus sabinoides*, *Juniperus virginiana*, *Juniperus communis* and *Juniperus ashei*); *Thuja* (e.g. *Thuja orientalis*); *Chamaecyparis* (e.g. *Chamaecyparis obtusa*); *Periplaneta* (e.g. *Periplaneta americana*); *Agropyron* (e.g. *Agropyron repens*); *Secale* (e.g. *Secale cereale*); *Triticum* (e.g. *Triticum aestivum*); *Dactylis* (e.g. *Dactylis glomerata*); *Festuca* (e.g. *Festuca elatior*); *Poa* (e.g. *Poa pratensis* or *Poa compressa*); *Avena* (e.g. *Avena sativa*); *Holcus* (e.g. *Holcus lanatus*); *Anthoxanthum* (e.g. *Anthoxanthum odoratum*); *Arrhenatherum* (e.g. *Arrhenatherum elatius*); *Agrostis* (e.g. *Agrostis alba*); *Phleum* (e.g. *Phleum pratense*); *Phalaris* (e.g. *Phalaris arundinacea*); *Paspalum* (e.g. *Paspalum notatum*); *Sorghum* (e.g. *Sorghum halepensis*); and *Bromus* (e.g. *Bromus inermis*).

A “subject having asthma” is a subject that has a disorder of the respiratory system characterized by inflammation, narrowing of the airways and increased reactivity of the

airways to inhaled agents. Asthma is frequently, although not exclusively associated with atopic or allergic symptoms. An “initiator” as used herein refers to a composition or environmental condition which triggers asthma. Initiators include, but are not limited to, allergens, cold temperatures, exercise, viral infections, SO<sub>2</sub>.

5 In another aspect the invention provides methods for treating or preventing asthma or allergy in a hypo-responsive subject. As used herein, a hypo-responsive subject is one who has previously failed to respond to a treatment directed at treating or preventing asthma or allergy or one who is at risk of not responding to such a treatment. The treatment directed at treating or preventing asthma or allergy may be an asthma/allergy medicament, in which case  
10 the hypo-responsive subject is one who is hypo-responsive to an asthma/allergy medicament.

Other subjects who are hypo-responsive include those who are refractory to an asthma/allergy medicament. As used herein, the term “refractory” means resistant or failure to yield to treatment. Such subjects may be those who never responded to an asthma/allergy medicament (i.e., subjects who are non-responders), or alternatively, they may be those who  
15 at one time responded to an asthma/allergy medicament, but have since that time have become refractory to the medicament. In some embodiments, the subject is one who is refractory to a subset of medicaments. A subset of medicaments is at least one medicament. In some embodiments, a subset refers to 2, 3, 4, 5, 6, 7, 8, 9, or 10 medicaments.

In other embodiments, hypo-responsive subjects are elderly subjects, regardless of  
20 whether they have or have not previously responded to a treatment directed at treating or preventing asthma or allergy. Elderly subjects, even those who have previously responded to such treatment, are considered to be at risk of not responding to a future administration of this treatment. Similarly, neonatal subjects are also considered to be at risk of not responding to treatment directed at treating or preventing asthma or allergy.

25 In some embodiments, an immunostimulatory nucleic acid is administered to the hypo-responsive subject without the further administration of an asthma/allergy medicament. In yet other embodiments, an asthma/allergy medicament is administered to the hypo-responsive subject, in which case it may be administered substantially simultaneously (i.e., concurrently) with, or following the administration of the immunostimulatory nucleic acid.

30 An “asthma/allergy medicament” as used herein is a composition of matter which reduces the symptoms, inhibits the asthmatic or allergic reaction, or prevents the development of an allergic or asthmatic reaction. Various types of medicaments for the treatment of asthma and allergy are described in the Guidelines For The Diagnosis and Management of

Asthma, Expert Panel Report 2, NIH Publication No. 97/4051, July 19, 1997, the entire contents of which are incorporated herein by reference. The summary of the medicaments as described in the NIH publication is presented below.

In most embodiments the asthma/allergy medicament is useful to some degree for treating both asthma and allergy. Some asthma/allergy medicaments are preferably used in combination with the immunostimulatory nucleic acids to treat asthma. These are referred to as asthma medicaments. Asthma medicaments include, but are not limited, PDE-4 inhibitors, bronchodilator/beta-2 agonists, K<sup>+</sup> channel openers, VLA-4 antagonists, neurokin antagonists, TXA<sub>2</sub> synthesis inhibitors, xanthanines, arachidonic acid antagonists, lipoxygenase inhibitors, thromboxin A<sub>2</sub> receptor antagonists, thromboxane A<sub>2</sub> antagonists, inhibitor of 5-lipoxygenase activation proteins, and protease inhibitors.

Bronchodilator/beta-2 agonists are a class of compounds which cause bronchodilation or smooth muscle relaxation. Bronchodilator/beta-2 agonists include, but are not limited to, salmeterol, salbutamol, albuterol, terbutaline, D2522/formoterol, fenoterol, bitolterol, pirbuerol methylxanthines and orciprenaline. Long-acting  $\beta_2$  agonists and bronchodilators are compounds which are used for long-term prevention of symptoms in addition to the anti-inflammatory therapies. They function by causing bronchodilation, or smooth muscle relaxation, following adenylate cyclase activation and increase in cyclic AMP producing functional antagonism of bronchoconstriction. These compounds also inhibit mast cell mediator release, decrease vascular permeability and increase mucociliary clearance. Long-acting  $\beta_2$  agonists include, but are not limited to, salmeterol and albuterol. These compounds are usually used in combination with corticosteroids and generally are not used without any inflammatory therapy. They have been associated with side effects such as tachycardia, skeletal muscle tremor, hypokalemia, and prolongation of QTc interval in overdose.

Methylxanthines, including for instance theophylline, have been used for long-term control and prevention of symptoms. These compounds cause bronchodilation resulting from phosphodiesterase inhibition and likely adenosine antagonism. It is also believed that these compounds may effect eosinophilic infiltration into bronchial mucosa and decrease T-lymphocyte numbers in the epithelium. Dose-related acute toxicities are a particular problem with these types of compounds. As a result, routine serum concentration must be monitored in order to account for the toxicity and narrow therapeutic range arising from individual differences in metabolic clearance. Side effects include tachycardia, nausea and vomiting, tachyarrhythmias, central nervous system stimulation, headache, seizures, hematemesis,



hyperglycemia and hypokalemia. Short-acting  $\beta_2$  agonists/bronchodilators relax airway smooth muscle, causing the increase in air flow. These types of compounds are a preferred drug for the treatment of acute asthmatic systems. Previously, short-acting  $\beta_2$  agonists had been prescribed on a regularly-scheduled basis in order to improve overall asthma symptoms.

5 Later reports, however, suggested that regular use of this class of drugs produced significant diminution in asthma control and pulmonary function (Sears, et al. *Lancet*; 336:1391-6, 1990). Other studies showed that regular use of some types of  $\beta_2$  agonists produced no harmful effects over a four-month period but also produced no demonstrable effects (Drazen, et al., *N. Eng. J. Med.*; 335:841-7, 1996). As a result of these studies, the daily use of short-acting  $\beta_2$  agonists is not generally recommended. Short-acting  $\beta_2$  agonists include, but are not limited to, albuterol, bitolterol, pirbuterol, and terbutaline. Some of the adverse effects associated with the mastration of short-acting  $\beta_2$  agonists include tachycardia, skeletal muscle tremor, hypokalemia, increased lactic acid, headache, and hyperglycemia.

Other asthma/allergy medicaments are preferably used in combination with the immunostimulatory nucleic acids to treat allergy. These are referred to as allergy medicaments. Allergy medicaments include, but are not limited to, anti-histamines, steroids, and prostaglandin inducers. Anti-histamines are compounds which counteract histamine released by mast cells or basophils. These compounds are well known in the art and commonly used for the treatment of allergy. Anti-histamines include, but are not limited to, loratidine, cetirizine, buclizine, ceterizine analogues, fexofenadine, terfenadine, desloratadine, norastemizole, epinastine, ebastine, ebastine, astemizole, levocabastine, azelastine, tranilast, terfenadine, mizolastine, betatastine, CS 560, and HSR 609. Prostaglandin inducers are compounds which induce prostaglandin activity. Prostaglandins function by regulating smooth muscle relaxation. Prostaglandin inducers include, but are not limited to, S-5751.

25 The asthma/allergy medicaments useful in combination with the immunostimulatory nucleic acids also include steroids and immunomodulators.

The steroids include, but are not limited to, beclomethasone, fluticasone, tramcinolone, budesonide, corticosteroids and budesonide. The combination of immunostimulatory nucleic acids and steroids are particularly well suited to the treatment of young subjects (e.g., children). To date, the use of steroids in children has been limited by the observation that some steroid treatments have been reportedly associated with growth retardation. Thus, according to the present invention, the immunostimulatory nucleic acids can be used in combination with growth retarding steroids, and can thereby provide a "steroid

sparing effect.” The combination of the two agents can result in lower required doses of steroids.

Corticosteroids are used long-term to prevent development of the symptoms, and suppress, control, and reverse inflammation arising from an initiator. Some corticosteroids can be administered by inhalation and others are administered systemically. The corticosteroids that are inhaled have an anti-inflammatory function by blocking late-reaction allergen and reducing airway hyper-responsiveness. These drugs also inhibit cytokine production, adhesion protein activation, and inflammatory cell migration and activation.

Corticosteroids include, but are not limited to, beclomethasone dipropionate, budesonide, flunisolide, fluticasone, propionate, and triamcinolone acetonide. Although dexamethasone is a corticosteroid having anti-inflammatory action, it is not regularly used for the treatment of asthma/allergy in an inhaled form because it is highly absorbed, it has long-term suppressive side effects at an effective dose. Dexamethasone, however, can be used according to the invention for the treating of asthma/allergy because when administered in combination with immunostimulatory nucleic acids it can be administered at a low dose to reduce the side effects. Additionally, the immunostimulatory nucleic acid can be administered to reduce the side effects of dexamethasone at higher concentrations. Some of the side effects associated with corticosteroid include cough, dysphonia, oral thrush (candidiasis), and in higher doses, systemic effects, such as adrenal suppression, osteoporosis, growth suppression, skin thinning and easy bruising. (Barnes & Peterson, *Am. Rev. Respir. Dis.*; 148:S1-S26, 1993; and Kamada et al., *Am. J. Respir. Crit. Care Med.*; 153:1739-48, 1996)

Systemic corticosteroids include, but are not limited to, methylprednisolone, prednisolone and prednisone. Corticosteroids are used generally for moderate to severe exacerbations to prevent the progression, reverse inflammation and speed recovery. These anti-inflammatory compounds include, but are not limited to, methylprednisolone, prednisolone, and prednisone. Corticosteroids are associated with reversible abnormalities in glucose metabolism, increased appetite, fluid retention, weight gain, mood alteration, hypertension, peptic ulcer, and rarely aseptic necrosis of femur. These compounds are useful for short-term (3-10 days) prevention of the inflammatory reaction in inadequately controlled persistent asthma. They also function in a long-term prevention of symptoms in severe persistent asthma to suppress and control and actually reverse inflammation. The side effects associated with systemic corticosteroids are even greater than those associated with inhaled

corticosteroids. Side effects include, for instance, reversible abnormalities in glucose metabolism, increased appetite, fluid retention, weight gain, mood alteration, hypertension, peptic ulcer and aseptic necrosis of femur, which are associated with short-term use. Some side effects associated with longer term use include adrenal axis suppression, growth  
5 suppression, dermal thinning, hypertension, diabetes, Cushing's syndrome, cataracts, muscle weakness, and in rare instances, impaired immune function. It is recommended that these types of compounds be used at their lowest effective dose (guidelines for the diagnosis and management of asthma; expert panel report to; NIH Publication No. 97-4051; July 1997). The inhaled corticosteroids are believed to function by blocking late reaction to allergen and  
10 reducing airway hyper-responsiveness. They are also believed to reverse  $\beta_2$ -receptor downregulation and to inhibit microvascular leakage.

The immunomodulators include, but are not limited to, the group consisting of anti-inflammatory agents, leukotriene antagonists, IL-4 muteins, soluble IL-4 receptors, immunosuppressants (such as tolerizing peptide vaccine), anti-IL-4 antibodies, IL-4  
15 antagonists, anti-IL-5 antibodies, soluble IL-13 receptor-Fc fusion proteins, anti-IL-9 antibodies, CCR3 antagonists, CCR5 antagonists, VLA-4 inhibitors, and , and downregulators of IgE.

Leukotriene modifiers are often used for long-term control and prevention of symptoms in mild persistent asthma. Leukotriene modifiers function as leukotriene receptor  
20 antagonists by selectively competing for LTD<sub>4</sub> and LTE<sub>4</sub> receptors. These compounds include, but are not limited to, zafirlukast tablets and zileuton tablets. Zileuton tablets function as 5-lipoxygenase inhibitors. These drugs have been associated with the elevation of liver enzymes and some cases of reversible hepatitis and hyperbilirubinemia. Leukotrienes are biochemical mediators that are released from mast cells, eosinophils, and basophils that  
25 cause contraction of airway smooth muscle and increase vascular permeability, mucous secretions and activate inflammatory cells in the airways of patients with asthma.

Other immunomodulators include neuropeptides that have been shown to have immunomodulating properties. Functional studies have shown that substance P, for instance, can influence lymphocyte function by specific receptor mediated mechanisms. Substance P  
30 also has been shown to modulate distinct immediate hypersensitivity responses by stimulating the generation of arachidonic acid-derived mediators from mucosal mast cells. J. McGillies, et al., Substance P and Immunoregulation, Fed. Proc. 46:196-9 (1987). Substance P is a neuropeptide first identified in 1931 by Von Euler and Gaddum. An unidentified depressor

substance in certain tissue extracts, J. Physiol. (London) 72:74-87 (1931). Its amino acid sequence, Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH<sub>2</sub> (Sequence Id. No. 1) was reported by Chang et al. in 1971. Amino acid sequence of substance P, Nature (London) New Biol. 232:86-87 (1971). The immunoregulatory activity of fragments of substance P has  
5 been studied by Siemion, et al. Immunoregulatory Activity of Substance P Fragments, Molec. Immunol. 27:887-890 (1990).

Another class of compounds is the down-regulators of IgE. These compounds include peptides or other molecules with the ability to bind to the IgE receptor and thereby prevent binding of antigen-specific IgE. Another type of downregulator of IgE is a monoclonal  
10 antibody directed against the IgE receptor-binding region of the human IgE molecule. Thus, one type of downregulator of IgE is an anti-IgE antibody or antibody fragment. Anti-IgE is being developed by Genentech. One of skill in the art could prepare functionally active antibody fragments of binding peptides which have the same function. Other types of IgE downregulators are polypeptides capable of blocking the binding of the IgE antibody to the Fc  
15 receptors on the cell surfaces and displacing IgE from binding sites upon which IgE is already bound.

One problem associated with downregulators of IgE is that many molecules don't have a binding strength to the receptor corresponding to the very strong interaction between the native IgE molecule and its receptor. The molecules having this strength tend to bind  
20 irreversibly to the receptor. However, such substances are relatively toxic since they can bind covalently and block other structurally similar molecules in the body. Of interest in this context is that the  $\alpha$  chain of the IgE receptor belongs to a larger gene family where i.e. several of the different IgG Fc receptors are contained. These receptors are absolutely essential for the defense of the body against i.e. bacterial infections. Molecules activated for  
25 covalent binding are, furthermore, often relatively unstable and therefore they probably have to be administered several times a day and then in relatively high concentrations in order to make it possible to block completely the continuously renewing pool of IgE receptors on mast cells and basophilic leukocytes.

These types of asthma/allergy medicaments are sometimes classified as long-term  
30 control medications or quick-relief medications. Long-term control medications include compounds such as corticosteroids (also referred to as glucocorticoids), methylprednisolone, prednisolone, prednisone, cromolyn sodium, nedocromil, long-acting  $\beta_2$ -agonists, methylxanthines, and leukotriene modifiers. Quick relief medications are useful for providing

quick relief of symptoms arising from allergic or asthmatic responses. Quick relief medications include short-acting  $\beta_2$  agonists, anticholinergics and systemic corticosteroids.

Chromolyn sodium and medocromil are used as long-term control medications for preventing primarily asthma symptoms arising from exercise or allergic symptoms arising from allergens. These compounds are believed to block early and late reactions to allergens by interfering with chloride channel function. They also stabilize mast cell membranes and inhibit activation and release of mediators from eosinophils and epithelial cells. A four to six week period of administration is generally required to achieve a maximum benefit.

Anticholinergics are generally used for the relief of acute bronchospasm. These compounds are believed to function by competitive inhibition of muscarinic cholinergic receptors. Anticholinergics include, but are not limited to, ipratropium bromide. These compounds reverse only cholinergically-mediated bronchospasm and do not modify any reaction to antigen. Side effects include drying of the mouth and respiratory secretions, increased wheezing in some individuals, blurred vision if sprayed in the eyes.

In addition to standard asthma/allergy medicaments other methods for treating asthma/allergy have been used either alone or in combination with established medicaments. One preferred, but frequently impossible, method of relieving allergies is allergen or initiator avoidance. Another method currently used for treating allergic disease involves the injection of increasing doses of allergen to induce tolerance to the allergen and to prevent further allergic reactions.

Allergen injection therapy (allergen immunotherapy) is known to reduce the severity of allergic rhinitis. This treatment has been theorized to involve the production of a different form of antibody, a protective antibody which is termed a "blocking antibody". Cooke, RA et al., Serologic Evidence of Immunity with Coexisting Sensitization in a Type of Human Allergy, Exp. Med. 62:733 (1935). Other attempts to treat allergy involve modifying the allergen chemically so that its ability to cause an immune response in the patient is unchanged, while its ability to cause an allergic reaction is substantially altered.

These methods, however, can take several years to be effective and are associated with the risk of side effects such as anaphylactic shock. The use of an immunostimulatory nucleic acid and asthma/allergy medicament in combination with an allergen avoids many of the side effects etc.

Commonly used allergy and asthma drugs which are currently in development or on the market are shown in Tables 1 and 2 respectively.

Table 1

| <b>Allergy Drugs in Development or on the Market</b> |                                                                                               |                            |
|------------------------------------------------------|-----------------------------------------------------------------------------------------------|----------------------------|
| MARKETER                                             | BRAND NAME (GENERIC NAME)                                                                     | MECHANISM                  |
| Schering-Plough                                      | Claritin + Claritin D (loratidine)                                                            | Anti-histamine             |
|                                                      | Vancenase (beclomethasone)                                                                    | Steroid                    |
| UCB                                                  | Reactine (cetirizine)(US)<br>Zyrtec (cetirizine)(ex US)                                       | Anti-histamine             |
|                                                      | Longifene (bucizine)                                                                          | Anti-histamine             |
|                                                      | UCB 28754 (ceterizine alologue)                                                               | Anti-histamine             |
| Glaxo                                                | Beconase (beclomethasone)                                                                     | Steroid                    |
|                                                      | Flonase (fluticasone)                                                                         | Steroid                    |
| Aventis                                              | Allegra (fexofenadine)                                                                        | Anti-histamine             |
|                                                      | Seldane (terfenadine)                                                                         | Anti-histamine             |
| Pfizer                                               | Reactine (cetirizine) (US)<br>Zyrtec/Reactine (cetirizine)(ex US)<br>(both licensed from UCB) | Anti-histamine             |
| Sepracor                                             | Allegra (fexofenadine)                                                                        | Anti-histamine             |
|                                                      | Desloratadine (lic to Schering-Plough)                                                        | Anti-histamine             |
|                                                      | Cetirizine (-) (lic to UCB)                                                                   | Anti-histamine             |
|                                                      | Norastemizole (option to J&J not exercised, 10-17-99)                                         | Anti-histamine             |
| B. Ingelheim                                         | Alesion (epinastine)                                                                          | Anti-histamine             |
| Aventis                                              | Kestin (ebastine) (US)<br>Bastel (ebastine ) (Eu/Ger)                                         | Anti-histamine             |
|                                                      | Nasacort (triamcinolone)                                                                      | Steroid                    |
| Johnson & Johnson                                    | Hismanol (astemizole)                                                                         | Anti-histamine             |
|                                                      | Livostin/Livocarb (levocabastine)                                                             | Anti-histamine             |
| AstraZeneca                                          | Rhinocort (budesonide) (Astra)                                                                | Steroid                    |
| Merck                                                | Rhinocort (budesonide)                                                                        | Steroid                    |
| Eisai                                                | Azeptin (azelastine)                                                                          | Anti-histamine             |
| Kissei                                               | Rizaben (tranilast)                                                                           | Anti-histamine             |
| Shionogi                                             | Triludan (terfenadine)                                                                        | Anti-histamine             |
|                                                      | S-5751                                                                                        | Prostaglandin inducer      |
| Schwarz                                              | Zolim (mizolastine)                                                                           | Anti-histamine             |
| Daiichi                                              | Zyrtec (cetirizine)                                                                           | Anti-histamine             |
| Tanabe Seiyaku                                       | Talion/TAU-284 (betatastine)                                                                  | Anti-histamine             |
| Sankyo**                                             | CS 560 (Hypersensitizaion therapy for cedar pollen allergy)                                   | Other                      |
| Asta Medica                                          | Azelastine-MDPI (azelastine)                                                                  | Anti-histamine             |
| BASF                                                 | HSR 609                                                                                       | Anti-histamine             |
| SR Pharma                                            | SRL 172                                                                                       | Immunomodulation           |
| Peptide Therapeutics                                 | Allergy vaccine (allergy (hayfever, anaphylaxis, atopic asthma)                               | Downregulates specific IgE |
|                                                      | Tolerizing peptide vaccine (rye grass peptide (T cell epitope))                               | Immuno-suppressant         |
| Coley Pharmaceutical                                 | CpG DNA                                                                                       | Immunomodulation           |

|           |          |                       |
|-----------|----------|-----------------------|
| Group     |          |                       |
| Genetech  | Anti-IgE | Down-regulator of IgE |
| SR Pharma | SRL 172  | Immunomodulation      |

Table 2

| <b><i>Asthma Drugs in Development or on the Market</i></b> |                                           |                                                   |
|------------------------------------------------------------|-------------------------------------------|---------------------------------------------------|
| MARKETER                                                   | BRAND NAME (GENERIC NAME)                 | MECHANISM                                         |
| Glaxo                                                      | Serevent (salmeterol)                     | Bronchodilator/beta-2 agonist                     |
|                                                            | Flovent (fluticasone)                     | Steroid                                           |
|                                                            | Flixotide (fluticasone)                   |                                                   |
|                                                            | Becotide (betamethasone)                  | Steroid                                           |
|                                                            | Ventolin (salbutamol)                     | Bronchodilator/beta-2 agonist                     |
|                                                            | Seretide (salmeterol + fluticasone)       | Beta agonist + steroid                            |
|                                                            | GW215864                                  | Steroid, hydolysable                              |
|                                                            | GW250495                                  | Steroid, hydolysable                              |
| AstraZeneca                                                | GW328267                                  | Adenosine A2 agonist                              |
|                                                            | Bambec (bambuterol) (Astra)               |                                                   |
|                                                            | Pulmicort (budesonide) (Astra)            | Steroid                                           |
|                                                            | Bricanyl Turbuhaler (terbutaline) (Astra) | Bronchodilator/beta-2 agonist                     |
|                                                            | Accolate (zafirlukast) (Zeneca)           | Leukotriene antagonist Slo-Phyllin (theophylline) |
|                                                            | Inspiryl (salbutamol) (Astra)             | Bronchodilator/beta-2 agonist                     |
|                                                            | Oxis Turbuhaler (D2522/formoterol)        | Bronchodilator/beta-2 agonist                     |
|                                                            | Symbicort (pulmicort-oxis combination)    | Steroid                                           |
|                                                            | Roflepanide (Astra)                       | Steroid                                           |
|                                                            | Bronica (seratrodast)                     | TXA2 synthesis inhibitor                          |
|                                                            | ZD 4407 (Zeneca)                          | 5 lipoxygenase inhibitor                          |
|                                                            | B. Ingelheim                              |                                                   |
|                                                            | Atrovent (ipratropium)                    | Bronchodilator/anti-cholinergic                   |
|                                                            | Berodual (ipratropium + fenoterol)        | Bronchodilator/anti-cholinergic                   |
|                                                            | Berotec (fenoterol)                       | Bronchodilator/beta-2 agonist                     |
| Schering-Plough                                            | Alupent (orciprenaline)                   | Bronchodilator/beta-2 agonist                     |
|                                                            | Ventilat (oxitropium)                     | Bronchodilator/anti-cholinergic                   |
|                                                            | Spiropent (clenbuterol)                   | Bronchodilator/beta-2 agonist                     |
|                                                            | Inhacort (flunisolide)                    | Steroid                                           |
|                                                            | BI679/tiotropium bromide                  |                                                   |
|                                                            | RPR 106541                                | Steroid                                           |
|                                                            | BIIX 1                                    | Potassium channel                                 |
|                                                            | BIIL284                                   | LTB-4 antagonist                                  |
|                                                            | Proventil (salbutamol)                    | Bronchodilator/beta-2 agonist                     |
|                                                            | Vanceril (beclomethasone)                 | Steroid                                           |
| RPR (Aventis)                                              | Mometasone furoate                        | Steroid                                           |
|                                                            | Theo-Dur (theophylline (w/ Astra)         |                                                   |
|                                                            | Uni-Dur (theophylline)                    |                                                   |
|                                                            | Asmanex (mometasone)                      | Steroid                                           |
|                                                            | CDP 835 (lic from Celltech)               | Anti-IL-5 Mab                                     |
|                                                            | Intal (disodium cromoglycate)             | Anti-inflammatory                                 |
|                                                            | Intal/Aarane (disodium cromoglycate)      |                                                   |
|                                                            | Tilade (nedocromil sodium)                | Anti-inflammatory                                 |
|                                                            | Azmacort (triamcinolone acetonide)        | Steroid                                           |

|                   |                                                                                                                                                                                                       |                                             |
|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|
|                   | RP 73401                                                                                                                                                                                              | PDE-4 inhibitor                             |
| Novartis          | Zaditen (ketotifen)                                                                                                                                                                                   | Anti-inflammatory                           |
|                   | Azmacort (triamcinolone)                                                                                                                                                                              | Steroid                                     |
|                   | Foradil (formoterol) (lic from Yamanouchi)                                                                                                                                                            | Bronchodilator/beta-2 agonist               |
|                   | E25                                                                                                                                                                                                   | Anti-IgE                                    |
|                   | KCO 912                                                                                                                                                                                               | K <sup>+</sup> channel opener               |
| Merck             | Singulair (montelukast)                                                                                                                                                                               | Leukotriene antagonist                      |
|                   | Pulmicort Turbuhaler (budesonide)                                                                                                                                                                     | Steroid                                     |
|                   | Slo-Phyllin (theophylline)                                                                                                                                                                            |                                             |
|                   | Symbicort (Pulmicort-Oxis combination)                                                                                                                                                                | Steroid                                     |
|                   | Oxis Turbuhaler (D2522/formoterol)                                                                                                                                                                    | Bronchodilator/beta-2 agonist               |
|                   | Roflepanide                                                                                                                                                                                           | Steroid                                     |
|                   | VLA-4 antagonist (lic from Biogen)                                                                                                                                                                    | VLA-4 antagonist                            |
| ONO               | Onon (pranlukast)                                                                                                                                                                                     | Leukotriene antagonist                      |
|                   | Vega (ozagrel)                                                                                                                                                                                        | TXA2 synthesis inhibitor                    |
| Fujisawa          | Intal (chromoglycate)                                                                                                                                                                                 | Anti-inflammatory                           |
|                   | FK 888                                                                                                                                                                                                | Neurokin antagonist                         |
| Forest Labs       | Aerobid (flunisolide)                                                                                                                                                                                 | Steroid                                     |
| IVAX              | Ventolin (salbutamol)                                                                                                                                                                                 | Bronchodilator/beta-2 agonist               |
|                   | Becotide (beclomethasone Easi-Breathe)                                                                                                                                                                | Steroid                                     |
|                   | Serevent (salmeterol)                                                                                                                                                                                 | Bronchodilator/beta-2 agonist               |
|                   | Flixotide (fluticasone)                                                                                                                                                                               | Steroid                                     |
|                   | Budesonide Dry Powder Inhaler                                                                                                                                                                         | Steroid                                     |
|                   | Salbutamol Dry Powder Inhaler                                                                                                                                                                         | Bronchodilator/beta-2 agonist               |
| Alza              | Volmax (salbutamol)                                                                                                                                                                                   | Bronchodilator/beta-2 agonist               |
| Altana            | Euphyllin (theophylline)                                                                                                                                                                              | Xanthanine                                  |
|                   | Ciclesonide                                                                                                                                                                                           | Arachidonic acid antagonist                 |
|                   | BY 217                                                                                                                                                                                                | PDE 4 inhibitor                             |
|                   | BY 9010N (ciclesonide)                                                                                                                                                                                | Steroid (nasal)                             |
| Tanabe<br>Seiyaku | Flucort (fluocinolone acetonide)                                                                                                                                                                      | Steroid                                     |
| Kissei            | Domenan (ozagrel)                                                                                                                                                                                     | TXA2 synthesis inhibitor                    |
| Abbott            | Zyflo (zileuton) (4X/day dosing, not competitive w/ Singulair or Accolate, no further interest in this area)                                                                                          | 5 lipoxygenase inhibitor                    |
| Asta Medica       | Aerobec (beclomethasone dipropionate) (w/ 3M)<br>Allergodil (azelastine)<br>Allergospasmin (sodium cromoglycate reproterol)<br>Bronchospasmin (reproterol)<br>Salbulair (salbutamol sulphate) (w/ 3M) |                                             |
|                   | TriNasal (triamcinolone)                                                                                                                                                                              | Steroid                                     |
|                   | Formoterol-MDPI                                                                                                                                                                                       | Beta 2 adrenoceptor agonist                 |
|                   | Budesonide-MDPI                                                                                                                                                                                       |                                             |
| UCB               | Atenos/Respecal (tulobuerol)                                                                                                                                                                          | Bronchodilator/beta-2 agonist               |
| Recordati         | Theodur (theophylline)                                                                                                                                                                                | Xanthine                                    |
| Medeva            | Clickhalers Asmasal, Asmabec (salbutamol beclomethasone dipropionate, dry inhaler)                                                                                                                    | Steroid                                     |
| Eisai             | E 6123                                                                                                                                                                                                | PAF receptor antagonist                     |
| Sankyo            | Zaditen (ketotifen)                                                                                                                                                                                   | Anti-inflammatory                           |
|                   | CS 615                                                                                                                                                                                                | Leukotriene antagonist                      |
| Shionogi          | Anboxan/S 1452 (domitroban)                                                                                                                                                                           | Thromboxin A2 receptor antagonist           |
| Yamanouchi        | YM 976                                                                                                                                                                                                | PDE 4 inhibitor                             |
|                   | YM 158                                                                                                                                                                                                | Leukotriene D4/thromboxan 2 dual antagonist |
| 3M Pharma         | Exirel (pirbuterol)                                                                                                                                                                                   |                                             |
| Hoechst           | Autoinhalers (3M albuterol projects)                                                                                                                                                                  | Bronchodilator/beta-2 agonist               |



|                                  |                                                            |                                                   |
|----------------------------------|------------------------------------------------------------|---------------------------------------------------|
| (Aventis)                        |                                                            |                                                   |
| SmithKline<br>Beecham            | Ariflo                                                     | PDE-4 inhibitor                                   |
|                                  | SB 240563                                                  | Anti-IL5 MAb (humanized)                          |
|                                  | SB 240683                                                  | Anti-IL4 Mab                                      |
|                                  | IDEC 151/clenoliximab                                      | Anti-CD4 MAb, primatised                          |
| Roche                            | Anti-IgE(GNE)/CGO51901                                     | Down-regulator of IgE                             |
| Sepracor                         | Fomoterol (R,R)                                            | Beta 2 adrenoceptor agonist                       |
|                                  | Xopenex (levalbuterol)                                     | Bet 2 adrenoceptor agonist                        |
| Bayer                            | BAY U 3405 (ramatroban)                                    | Thromboxane A2 antagonist                         |
|                                  | BAY 16-9996 (once monthly dosing)                          | IL4 mutein                                        |
|                                  | BAY 19-8004                                                | PDE-4 inhibitor                                   |
| SR Pharma                        | SRL 172                                                    | Immunomodulation                                  |
| Immunex                          | Nuvance                                                    | Soluble IL-4 receptor<br>(immunomodulator)        |
| Biogen                           | Anti-VLA-4                                                 | Immunosuppressant                                 |
| Vanguard                         | VML 530                                                    | Inhibitor of 5-lipoxygenase activation<br>protein |
| Recordati                        | Respix (zafirlukast)                                       | Leukotriene antagonist                            |
| Genentech                        | Anti-IgE MAb                                               | Down-regulator of IgE                             |
| Warner<br>Lambert                | CI-1018                                                    | PDE 4 inhibitor                                   |
| Celltech/<br>Chiroscience        | CDP 835/SCH 55700 (anti-IL-5) (lic to Schering-<br>Plough) | IL-5 antagonist Mab                               |
|                                  | D 4418 (w/ Schering-Plough)                                | PDE 4 inhibitor                                   |
|                                  | CDP 840 (Celltech)                                         | PDE 4 inhibitor                                   |
| AHP                              | Pda-641 (asthma steroid replacement)                       |                                                   |
| Peptide<br>Therapeutics          | RAPID Technology Platform                                  | Protease inhibitors                               |
| Coley<br>Pharmaceutical<br>Group | CpG DNA                                                    | Immunomodulation                                  |

In some cases the subject is exposed to an allergen in addition to being treated with the immunostimulatory nucleic acid and the asthma/allergy medicament. In this case the subject is said to be exposed to the allergen. As used herein, the term "exposed to" refers to either the active step of contacting the subject with an allergen or the passive exposure of the subject to the allergen *in vivo*. Methods for the active exposure of a subject to an allergen are well-known in the art. In general, an allergen is administered directly to the subject by any means such as intravenous, intramuscular, oral, transdermal, mucosal, intranasal, intratracheal, or subcutaneous administration. The allergen can be administered systemically or locally.

Methods for administering the allergen and the immunostimulatory nucleic acid/asthma/allergy medicament are described in more detail below. A subject is passively exposed to an allergen if an allergen becomes available for exposure to the immune cells in the body. A subject may be passively exposed to an allergen, for instance, by entry of an

allergen into the body when the allergen is present in the environment surrounding the subject, i.e. pollen.

The methods in which a subject is passively exposed to an allergen can be particularly dependent on timing of administration of the immunostimulatory nucleic acid and the  
5 asthma/allergy medicament. For instance, in a subject at risk of developing an allergic or asthmatic response, the subject may be administered the immunostimulatory nucleic acid and the asthma/allergy medicament on a regular basis when that risk is greatest, i.e., during pollen allergy season. Additionally the immunostimulatory nucleic acid and the asthma/allergy medicament may be administered to travelers before they travel to a destination where they  
10 are at risk of exposure to a particular allergen.

As used herein, the term “prevent”, “prevented”, or “preventing” when used with respect to the treatment of an allergic or asthmatic disorder refers to a prophylactic treatment which increases the resistance of a subject to an allergen or initiator or, in other words, decreases the likelihood that the subject will develop an allergic or asthmatic response to the  
15 allergen or initiator as well as a treatment after the allergic or asthmatic disorder has begun in order to fight the allergy/asthma, e.g., reduce or eliminate it altogether or prevent it from becoming worse.

The term “substantially purified” as used herein refers to a molecular species which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is  
20 naturally associated. One skilled in the art can purify allergenic polypeptides using standard techniques for protein purification. The substantially pure polypeptide will often yield a single major band on a non-reducing polyacrylamide gel. In the case of partially glycosylated polypeptides or those that have several start codons, there may be several bands on a non-reducing polyacrylamide gel, but these will form a distinctive pattern for that polypeptide.  
25 The purity of the allergenic polypeptide can also be determined by amino-terminal amino acid sequence analysis.

The allergen and/or polypeptide asthma/allergy medicament may be in the form of a polypeptide when administered to the subject or it may be encoded by a nucleic acid vector. If the nucleic acid vector is administered to the subject the protein is expressed *in vivo*. Minor  
30 modifications of the primary amino acid sequences of polypeptide allergens may also result in a polypeptide which has substantially equivalent allergenic activity as compared to the unmodified counterpart polypeptide. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous.

The nucleic acid encoding the allergen or asthma/allergy medicament is operatively linked to a gene expression sequence which directs the expression of the protein within a eukaryotic cell. The "gene expression sequence" is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer combination, which facilitates the efficient transcription and translation of the protein which it is operatively linked. The gene expression sequence may, for example, be a mammalian or viral promoter, such as a constitutive or inducible promoter. Constitutive mammalian promoters include, but are not limited to, the promoters for the following genes: hypoxanthine phosphoribosyl transferase (HPTR), adenosine deaminase, pyruvate kinase, b-actin promoter and other constitutive promoters. Exemplary viral promoters which function constitutively in eukaryotic cells include, for example, promoters from the cytomegalovirus (CMV), simian virus (e.g., SV40), papilloma virus, adenovirus, human immunodeficiency virus (HIV), Rous sarcoma virus, cytomegalovirus, the long terminal repeats (LTR) of Moloney leukemia virus and other retroviruses, and the thymidine kinase promoter of herpes simplex virus. Other constitutive promoters are known to those of ordinary skill in the art. The promoters useful as gene expression sequences of the invention also include inducible promoters. Inducible promoters are expressed in the presence of an inducing agent. For example, the metallothionein promoter is induced to promote transcription and translation in the presence of certain metal ions. Other inducible promoters are known to those of ordinary skill in the art.

In general, the gene expression sequence shall include, as necessary, 5' non-transcribing and 5' non-translating sequences involved with the initiation of transcription and translation, respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. Especially, such 5' non-transcribing sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined antigen nucleic acid. The gene expression sequences optionally include enhancer sequences or upstream activator sequences as desired.

As used herein, the nucleic acid sequence encoding the protein and the gene expression sequence are said to be "operably linked" when they are covalently linked in such a way as to place the expression or transcription and/or translation of the antigen coding sequence under the influence or control of the gene expression sequence. Two DNA sequences are said to be operably linked if induction of a promoter in the 5' gene expression sequence results in the transcription of the gene sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift

mutation, (2) interfere with the ability of the promoter region to direct the transcription of the antigen sequence, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a gene expression sequence would be operably linked to a specific nucleic acid sequence if the gene expression sequence were capable of effecting transcription of that nucleic acid sequence such that the resulting transcript is translated into the desired protein or polypeptide.

The immunostimulatory nucleic acids may also be delivered to the subject in the form of a plasmid vector. In some embodiments, one plasmid vector could include both the immunostimulatory nucleic acid and a nucleic acid encoding a protein asthma/allergy medicament and/or an allergen. In other embodiments, separate plasmids could be used. In yet other embodiments, no plasmids could be used.

The compositions of the invention may be delivered to the immune system or other target cells alone or in association with a vector. In its broadest sense, a "vector" is any vehicle capable of facilitating the transfer of the compositions to the target cells. The vector generally transports the nucleic acid to the immune cells with reduced degradation relative to the extent of degradation that would result in the absence of the vector.

In general, the vectors useful in the invention are divided into two classes: biological vectors and chemical/physical vectors. Biological vectors and chemical/physical vectors are useful for delivery/uptake of nucleic acids, asthma/allergy medicaments, and/or allergens to/by a target cell.

Biological vectors include, but are not limited to, plasmids, phagemids, viruses, other vehicles derived from viral or bacterial sources that have been manipulated by the insertion or incorporation of nucleic acid sequences, and free nucleic acid fragments which can be attached to nucleic acid sequences. Viral vectors are a preferred type of biological vector and include, but are not limited to, nucleic acid sequences from the following viruses: retroviruses, such as: Moloney murine leukemia virus; Harvey murine sarcoma virus; murine mammary tumor virus; Rous sarcoma virus; adenovirus; adeno-associated virus; SV40-type viruses; polyoma viruses; Epstein-Barr viruses; papilloma viruses; herpes viruses; vaccinia viruses; polio viruses; and RNA viruses such as any retrovirus. One can readily employ other viral vectors not named but known in the art.

Preferred viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with a nucleic acid of interest. Non-cytopathic viruses include retroviruses, the life cycle of which involves reverse transcription of genomic viral

RNA into DNA with subsequent proviral integration into host cellular DNA. Retroviruses have been approved for human gene therapy trials. In general, the retroviruses are replication-deficient (i.e., capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression  
5 vectors have general utility for the high-efficiency transduction of genes *in vivo*. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell lined with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are  
10 provided in Kriegler, M., "Gene Transfer and Expression, A Laboratory Manual," W.H. Freeman Co., New York (1990) and Murry, E.J. Ed. "Methods in Molecular Biology," vol. 7, Humana Press, Inc., Clifton, New Jersey (1991).

Another preferred virus for certain applications is the adeno-associated virus, a double-stranded DNA virus. The adeno-associated virus can be engineered to be replication -  
15 deficient and is capable of infecting a wide range of cell types and species. It further has advantages, such as heat and lipid solvent stability; high transduction frequencies in cells of diverse lineages; and lack of superinfection inhibition thus allowing multiple series of transductions. Reportedly, the adeno-associated virus can integrate into human insertional mutagenesis and variability of inserted gene expression. In addition, wild-type adeno-  
20 associated virus infections have been followed in tissue culture for greater than 100 passages in the absence of selective pressure, implying that the adeno-associated virus genomic integration is a relatively stable event. The adeno-associated virus can also function in an extrachromosomal fashion.

Other biological vectors include plasmid vectors. Plasmid vectors have been  
25 extensively described in the art and are well-known to those of skill in the art. See e.g., Sambrook et al., "Molecular Cloning: A Laboratory Manual, "Second Edition, Cold Spring Harbor Laboratory Press, 1989. In the last few years, plasmid vectors have been found to be particularly advantageous for delivering genes to cells *in vivo* because of their inability to replicate within and integrate into a host genome. These plasmids, however, having a  
30 promoter compatible with the host cell, can express a peptide from a gene operatively encoded within the plasmid. Some commonly used plasmids include pBR322, pUC18, pUC19, pRC/CMV, SV40, and pBlueScript. Other plasmids are well-known to those of

ordinary skill in the art. Additionally, plasmids may be custom designed using restriction enzymes and ligation reactions to remove and add specific fragments of DNA.

It has recently been discovered that gene carrying plasmids can be delivered to the immune system using bacteria. Modified forms of bacteria such as *Salmonella* can be transfected with the plasmid and used as delivery vehicles. The bacterial delivery vehicles can be administered to a host subject orally or by other administration means. The bacteria deliver the plasmid to immune cells, e.g. B cells, dendritic cells, likely by passing through the gut barrier. High levels of immune protection have been established using this methodology. Such methods of delivery are useful for the aspects of the invention utilizing systemic delivery of allergen, immunostimulatory nucleic acid and/or other therapeutic agent.

In addition to the biological vectors, chemical/physical vectors may be used to deliver a nucleic acid, asthma/allergy medicament, and/or allergen to a target cell and facilitate uptake thereby. As used herein, a "chemical/physical vector" refers to a natural or synthetic molecule, other than those derived from bacteriological or viral sources, capable of delivering the nucleic acid, asthma/allergy medicament, and/or allergen to a cell.

A preferred chemical/physical vector of the invention is a colloidal dispersion system. Colloidal dispersion systems include lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system of the invention is a liposome. Liposomes are artificial membrane vessels which are useful as a delivery vector *in vivo* or *in vitro*. It has been shown that large unilamellar vessels (LUV), which range in size from 0.2 - 4.0  $\mu\text{m}$  can encapsulate large macromolecules. RNA, DNA, and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, et al., *Trends Biochem. Sci.*, (1981) 6:77).

Liposomes may be targeted to a particular tissue by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein. Ligands which may be useful for targeting a liposome to an immune cell include, but are not limited to: intact or fragments of molecules which interact with immune cell specific receptors and molecules, such as antibodies, which interact with the cell surface markers of immune cells. Such ligands may easily be identified by binding assays well known to those of skill in the art. Additionally, the vector may be coupled to a nuclear targeting peptide, which will direct the vector to the nucleus of the host cell.

Lipid formulations for transfection are commercially available from QIAGEN, for example, as EFFECTENE™ (a non-liposomal lipid with a special DNA condensing enhancer) and SUPERFECT™ (a novel acting dendrimeric technology).

Liposomes are commercially available from Gibco BRL, for example, as LIPOFECTIN™ and LIPOFECTACE™, which are formed of cationic lipids such as N-[1-(2, 3 dioleyloxy)-propyl]-N, N, N-trimethylammonium chloride (DOTMA) and dimethyl dioctadecylammonium bromide (DDAB). Methods for making liposomes are well known in the art and have been described in many publications. Liposomes also have been reviewed by Gregoriadis, G. in *Trends in Biotechnology*, (1985) 3:235-241.

In one embodiment, the vehicle is a biocompatible microparticle or implant that is suitable for implantation or administration to the mammalian recipient. Exemplary bioerodible implants that are useful in accordance with this method are described in PCT International application no. PCT/US/03307 (Publication No. WO95/24929, entitled "Polymeric Gene Delivery System". PCT/US/0307 describes a biocompatible, preferably biodegradable polymeric matrix for containing an exogenous gene under the control of an appropriate promoter. The polymeric matrix can be used to achieve sustained release of the exogenous gene in the patient.

The polymeric matrix preferably is in the form of a microparticle such as a microsphere (wherein the a nucleic acid, asthma/allergy medicament, and/or allergen is dispersed throughout a solid polymeric matrix) or a microcapsule (wherein the a nucleic acid, asthma/allergy medicament, and/or allergen is stored in the core of a polymeric shell). Other forms of the polymeric matrix for containing the a nucleic acid, asthma/allergy medicament, and/or allergen include films, coatings, gels, implants, and stents. The size and composition of the polymeric matrix device is selected to result in favorable release kinetics in the tissue into which the matrix is introduced. The size of the polymeric matrix further is selected according to the method of delivery which is to be used, typically injection into a tissue or administration of a suspension by aerosol into the nasal and/or pulmonary areas. Preferably when an aerosol route is used the polymeric matrix and the nucleic acid, asthma/allergy medicament, and/or allergen are encompassed in a surfactant vehicle. The polymeric matrix composition can be selected to have both favorable degradation rates and also to be formed of a material which is bioadhesive, to further increase the effectiveness of transfer when the matrix is administered to a nasal and/or pulmonary surface that has sustained an injury. The

matrix composition also can be selected not to degrade, but rather, to release by diffusion over an extended period of time.

In another embodiment the chemical/physical vector is a biocompatible microsphere that is suitable for delivery, such as oral or mucosal delivery. Such microspheres are disclosed in Chickering et al., *Biotech. And Bioeng.*, (1996) 52:96-101 and Mathiowitz et al., *Nature*, (1997) 386:410-414 and PCT Patent Application WO97/03702.

Both non-biodegradable and biodegradable polymeric matrices can be used to deliver the nucleic acid, asthma/allergy medicament, and/or allergen to the subject. Biodegradable matrices are preferred. Such polymers may be natural or synthetic polymers. The polymer is selected based on the period of time over which release is desired, generally in the order of a few hours to a year or longer. Typically, release over a period ranging from between a few hours and three to twelve months is most desirable. The polymer optionally is in the form of a hydrogel that can absorb up to about 90% of its weight in water and further, optionally is cross-linked with multi-valent ions or other polymers.

Bioadhesive polymers of particular interest include bioerodible hydrogels described by H.S. Sawhney, C.P. Pathak and J.A. Hubell in *Macromolecules*, (1993) 26:581-587, the teachings of which are incorporated herein, polyhyaluronic acids, casein, gelatin, glutin, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl methacrylates), poly(ethyl methacrylates), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate).

Compaction agents also can be used alone, or in combination with, a biological or chemical/physical vector. A "compaction agent", as used herein, refers to an agent, such as a histone, that neutralizes the negative charges on the nucleic acid and thereby permits compaction of the nucleic acid into a fine granule. Compaction of the nucleic acid facilitates the uptake of the nucleic acid by the target cell. The compaction agents can be used alone, i.e., to deliver a nucleic acid in a form that is more efficiently taken up by the cell or, more preferably, in combination with one or more of the above-described vectors.

Other exemplary compositions that can be used to facilitate uptake by a target cell of the nucleic acid, asthma/allergy medicament, and/or allergen include calcium phosphate and other chemical mediators of intracellular transport, microinjection compositions,



electroporation and homologous recombination compositions (e.g., for integrating a nucleic acid into a preselected location within the target cell chromosome).

The immunostimulatory nucleic acid and/or the asthma/allergy medicament the antigen and/or other therapeutics may be administered alone (e.g. in saline or buffer) or using any delivery vectors known in the art. For instance the following delivery vehicles have been described: Cochleates (Gould-Fogerite et al., 1994, 1996); Emulsomes (Vancott et al., 1998, Lowell et al., 1997); ISCOMs (Mowat et al., 1993, Carlsson et al., 1991, Hu et., 1998, Morein et al., 1999); Liposomes (Childers et al., 1999, Michalek et al., 1989, 1992, de Haan 1995a, 1995b); Live bacterial vectors (e.g., *Salmonella*, *Escherichia coli*, *Bacillus calmatte-guerin*, *Shigella*, *Lactobacillus*) (Hone et al., 1996, Pouwels et al., 1998, Chatfield et al., 1993, Stover et al., 1991, Nugent et al., 1998); Live viral vectors (e.g., Vaccinia, adenovirus, Herpes Simplex) (Gallichan et al., 1993, 1995, Moss et al., 1996, Nugent et al., 1998, Flexner et al., 1988, Morrow et al., 1999); Microspheres (Gupta et al., 1998, Jones et al., 1996, Maloy et al., 1994, Moore et al., 1995, O'Hagan et al., 1994, Eldridge et al., 1989); Nucleic acid vaccines (Fynan et al., 1993, Kuklin et al., 1997, Sasaki et al., 1998, Okada et al., 1997, Ishii et al., 1997); Polymers (e.g. carboxymethylcellulose, chitosan) (Hamajima et al., 1998, Jabbal-Gill et al., 1998); Polymer rings (Wyatt et al., 1998); Proteosomes (Vancott et al., 1998, Lowell et al., 1988, 1996, 1997); Sodium Fluoride (Hashi et al., 1998); Transgenic plants (Tacket et al., 1998, Mason et al., 1998, Haq et al., 1995); Virosomes (Gluck et al., 1992, Mengiardi et al., 1995, Cryz et al., 1998); Virus-like particles (Jiang et al., 1999, Leibl et al., 1998).

The immunostimulatory nucleic acid and asthma/allergy medicament can be combined with other therapeutic agents such as adjuvants to enhance immune responses even further. The immunostimulatory nucleic acid, asthma/allergy medicament and other therapeutic agent may be administered simultaneously or sequentially. When the other therapeutic agents are administered simultaneously they can be administered in the same or separate formulations, but are administered at the same time. The other therapeutic agents are administered sequentially with one another and with the immunostimulatory nucleic acid and asthma/allergy medicament, when the administration of the other therapeutic agents and the immunostimulatory nucleic acid and asthma/allergy medicament is temporally separated. The separation in time between the administration of these compounds may be a matter of minutes or it may be longer. Other therapeutic agents include but are not limited to non-nucleic acid adjuvants, cytokines, antibodies, antigens, etc.

A “non-nucleic acid adjuvant” is any molecule or compound except for the immunostimulatory nucleic acids described herein which can stimulate the humoral and/or cellular immune response. Non-nucleic acid adjuvants include, for instance, adjuvants that create a depo effect, immune stimulating adjuvants, adjuvants that create a depo effect and stimulate the immune system and mucosal adjuvants.

An “adjuvant that creates a depo effect” as used herein is an adjuvant that causes an antigen or allergen to be slowly released in the body, thus prolonging the exposure of immune cells to the antigen or allergen. This class of adjuvants includes but is not limited to alum (e.g., aluminum hydroxide, aluminum phosphate); or emulsion-based formulations including mineral oil, non-mineral oil, water-in-oil or oil-in-water-in oil emulsion, oil-in-water emulsions such as Seppic ISA series of Montanide adjuvants (e.g., Montanide ISA 720, AirLiquide, Paris, France); MF-59 (a squalene-in-water emulsion stabilized with Span 85 and Tween 80; Chiron Corporation, Emeryville, CA; and PROVAX (an oil-in-water emulsion containing a stabilizing detergent and a micelle-forming agent; IDEC, Pharmaceuticals Corporation, San Diego, CA).

An “immune stimulating adjuvant” is an adjuvant that causes activation of a cell of the immune system. It may, for instance, cause an immune cell to produce and secrete cytokines. This class of adjuvants includes but is not limited to saponins purified from the bark of the *Q. saponaria* tree, such as QS21 (a glycolipid that elutes in the 21<sup>st</sup> peak with HPLC fractionation; Aquila Biopharmaceuticals, Inc., Worcester, MA); poly[di(carboxylatophenoxy)phosphazene (PCPP polymer; Virus Research Institute, USA); derivatives of lipopolysaccharides such as monophosphoryl lipid A (MPL; RibImmunoChem Research, Inc., Hamilton, MT), muramyl dipeptide (MDP; Rib) and threonylmuramyl dipeptide (t-MDP; Rib); OM-174 (a glucosamine disaccharide related to lipid A; OM Pharma SA, Meyrin, Switzerland); and Leishmania elongation factor (a purified *Leishmania* protein; Corixa Corporation, Seattle, WA).

“Adjuvants that create a depo effect and stimulate the immune system” are those compounds which have both of the above-identified functions. This class of adjuvants includes but is not limited to ISCOMS (Immunostimulating complexes which contain mixed saponins, lipids and form virus-sized particles with pores that can hold antigen; CSL, Melbourne, Australia); SB-AS2 (SmithKline Beecham adjuvant system #2 which is an oil-in-water emulsion containing MPL and QS21: SmithKline Beecham Biologicals [SBB], Rixensart, Belgium); SB-AS4 (SmithKline Beecham adjuvant system #4 which contains alum

and MPL; SBB, Belgium); non-ionic block copolymers that form micelles such as CRL 1005 (these contain a linear chain of hydrophobic polyoxpropylene flanked by chains of polyoxyethylene; Vaxcel, Inc., Norcross, GA); and Syntex Adjuvant Formulation (SAF, an oil-in-water emulsion containing Tween 80 and a nonionic block copolymer; Syntex Chemicals, Inc., Boulder, CO).

A "non-nucleic acid mucosal adjuvant" as used herein is an adjuvant other than an immunostimulatory nucleic acid that is capable of inducing a mucosal immune response in a subject when administered to a mucosal surface in conjunction with an antigen or allergen. Mucosal adjuvants include but are not limited to Bacterial toxins: e.g., Cholera toxin (CT), CT derivatives including but not limited to CT B subunit (CTB) (Wu et al., 1998, Tochikubo et al., 1998); CTD53 (Val to Asp) (Fontana et al., 1995); CTK97 (Val to Lys) (Fontana et al., 1995); CTK104 (Tyr to Lys) (Fontana et al., 1995); CTD53/K63 (Val to Asp, Ser to Lys) (Fontana et al., 1995); CTH54 (Arg to His) (Fontana et al., 1995); CTN107 (His to Asn) (Fontana et al., 1995); CTE114 (Ser to Glu) (Fontana et al., 1995); CTE112K (Glu to Lys) (Yamamoto et al., 1997a); CTS61F (Ser to Phe) (Yamamoto et al., 1997a, 1997b); CTS106 (Pro to Lys) (Douce et al., 1997, Fontana et al., 1995); and CTK63 (Ser to Lys) (Douce et al., 1997, Fontana et al., 1995), Zonula occludens toxin, zot, Escherichia coli heat-labile enterotoxin, Labile Toxin (LT), LT derivatives including but not limited to LT B subunit (LTB) (Verweij et al., 1998); LT7K (Arg to Lys) (Komase et al., 1998, Douce et al., 1995); LT61F (Ser to Phe) (Komase et al., 1998); LT112K (Glu to Lys) (Komase et al., 1998); LT118E (Gly to Glu) (Komase et al., 1998); LT146E (Arg to Glu) (Komase et al., 1998); LT192G (Arg to Gly) (Komase et al., 1998); LTK63 (Ser to Lys) (Marchetti et al., 1998, Douce et al., 1997, 1998, Di Tommaso et al., 1996); and LTR72 (Ala to Arg) (Giuliani et al., 1998), Pertussis toxin, PT. (Lycke et al., 1992, Spangler BD, 1992, Freytag and Clements, 1999, Roberts et al., 1995, Wilson et al., 1995) including PT-9K/129G (Roberts et al., 1995, Copley et al., 1995); Toxin derivatives (see below) (Holmgren et al., 1993, Verweij et al., 1998, Rappuoli et al., 1995, Freytag and Clements, 1999); Lipid A derivatives (e.g., monophosphoryl lipid A, MPL) (Sasaki et al., 1998, Vancott et al., 1998; Muramyl Dipeptide (MDP) derivatives (Fukushima et al., 1996, Ogawa et al., 1989, Michalek et al., 1983, Morisaki et al., 1983); Bacterial outer membrane proteins (e.g., outer surface protein A (OspA) lipoprotein of *Borrelia burgdorferi*, outer membrane protine of *Neisseria meningitidis*) (Marinaro et al., 1999, Van de Verg et al., 1996); Oil-in-water emulsions (e.g., MF59) (Barchfield et al., 1999, Verschoor et al., 1999, O'Hagan, 1998); Aluminum salts

(Isaka et al., 1998, 1999); and Saponins (e.g., QS21) Aquila Biopharmaceuticals, Inc.,  
Worster, MA) (Sasaki et al., 1998, MacNeal et al., 1998), ISCOMS, MF-59 (a squalene-in-  
water emulsion stabilized with Span 85 and Tween 80; Chiron Corporation, Emeryville, CA);  
the Seppic ISA series of Montanide adjuvants (e.g., Montanide ISA 720; AirLiquide, Paris,  
5 France); PROVAX (an oil-in-water emulsion containing a stabilizing detergent and a micell-  
forming agent; IDEC Pharmaceuticals Corporation, San Diego, CA); Syntex Adjuvant  
Formulation (SAF; Syntex Chemicals, Inc., Boulder, CO);  
poly[di(carboxylatophenoxy)phosphazene (PCPP polymer; Virus Research Institute, USA)  
and Leishmania elongation factor (Corixa Corporation, Seattle, WA).

10 Immune responses can also be induced or augmented by the co-administration or co-  
linear expression of cytokines (Bueler & Mulligan, 1996; Chow *et al.*, 1997; Geissler *et*  
*al.*, 1997; Iwasaki *et al.*, 1997; Kim *et al.*, 1997) or B-7 co-stimulatory molecules (Iwasaki *et*  
*al.*, 1997; Tsuji *et al.*, 1997) with the immunostimulatory nucleic acids and asthma/allergy  
medicaments. The cytokines can be administered directly with immunostimulatory nucleic  
15 acids or may be administered in the form of a nucleic acid vector that encodes the cytokine,  
such that the cytokine can be expressed *in vivo*. In one embodiment, the cytokine is  
administered in the form of a plasmid expression vector. The term "cytokine" is used as a  
generic name for a diverse group of soluble proteins and peptides which act as humoral  
regulators at nano- to picomolar concentrations and which, either under normal or  
20 pathological conditions, modulate the functional activities of individual cells and tissues.  
These proteins also mediate interactions between cells directly and regulate processes taking  
place in the extracellular environment. Examples of cytokines include, but are not limited to  
IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-15, IL-18 granulocyte-macrophage colony  
stimulating factor (GM-CSF), granulocyte colony stimulating factor (GCSF), interferon- $\gamma$  ( $\gamma$ -  
25 IFN), IFN- $\alpha$ , tumor necrosis factor (TNF), TGF- $\beta$ , FLT-3 ligand, and CD40 ligand.  
Cytokines play a role in directing the T cell response. Helper (CD4+) T cells orchestrate the  
immune response of mammals through production of soluble factors that act on other immune  
system cells, including other T cells. Most mature CD4+ T helper cells express one of two  
cytokine profiles: Th1 or Th2. In some embodiments it is preferred that the cytokine be a Th1  
30 cytokine.

The term "effective amount" of an immunostimulatory nucleic acid and an  
asthma/allergy medicament refers to the amount necessary or sufficient to realize a desired  
biologic effect. For example, an effective amount of an immunostimulatory nucleic acid and

an asthma/allergy medicament for treating or preventing asthma or preventing is that amount necessary to prevent the development of IgE in response to an allergen or initiator upon exposure to the allergen or initiator is that amount necessary to cause the shift from Th2 to Th1 response in response to an allergen or initiator.

5 Combined with the teachings provided herein, by choosing among the various active compounds and weighing factors such as potency, relative bioavailability, patient body weight, severity of adverse side-effects and preferred mode of administration, an effective prophylactic or therapeutic treatment regimen can be planned which does not cause substantial toxicity and yet is entirely effective to treat the particular subject. The effective  
10 amount for any particular application can vary depending on such factors as the disease or condition being treated, the particular immunostimulatory nucleic acid or asthma/allergy medicament being administered (e.g. the type of nucleic acid, i.e. a CpG nucleic acid, the number of unmethylated CpG motifs or their location in the nucleic acid, the degree of modification of the backbone to the oligonucleotide the type of medicament), the size of the  
15 subject, or the severity of the disease or condition. One of ordinary skill in the art can empirically determine the effective amount of a particular immunostimulatory nucleic acid and/or asthma/allergy medicament and/or other therapeutic agent without necessitating undue experimentation.

Depending upon the aspect of the invention, the immunostimulatory nucleic acid and  
20 asthma/allergy medicament may be administered in a synergistic amount effective to treat or prevent asthma or allergy. A synergistic amount is that amount which produces a physiological response that is greater than the sum of the individual effects of either the immunostimulatory nucleic acid or the asthma/allergy medicament alone. For instance, in  
25 some embodiments of the invention, the physiological effect is a reduction in IgE levels. A synergistic amount is that amount which produces a reduction in IgE that is greater than the sum of the IgE reduced by either the immunostimulatory nucleic acid or the asthma/allergy medicament alone. In other embodiments, the physiological result is a shift from Th2  
cytokines, such as IL-4 and IL-5, to Th1 cytokines, such as IFN- $\gamma$  and IL-12. The synergistic  
amount in this case is that amount which produces the shift to a Th1 cytokine that is greater  
30 than the sum of the shift produced by either the immunostimulatory nucleic acid or the asthma/allergy medicament alone. In other embodiments the physiological result is a decrease in eosinophilia, hyperreactivity, or lung function.

In some embodiments of the invention, the immunostimulatory nucleic acid is administered in an effective amount for preventing bacterial or viral infection.

Immunostimulatory nucleic acids are known to be useful for preventing bacterial and viral infections. Bacterial and viral infections exacerbate and/or induce allergy and/or asthma. In this aspect of the invention, the immunostimulatory nucleic acid is administered to the subject in an amount effective to prevent bacterial and viral infection and the asthma/allergy medicament is administered to the subject when symptoms of allergy or asthma appear. Thus, the immunostimulatory nucleic acid is administered to the subject and then the asthma/allergy medicament is subsequently administered to the subject or they are administered together at the same time. This method is particularly useful in subjects such as children and immunocompromised subjects, or elderly subjects, who are particularly susceptible to bacterial or viral disease.

In aspects of the invention directed at treating subjects in anticipation of an asthmatic or allergic event or season (e.g., in anticipation of the hay-fever season), the subjects may be administered an immunostimulatory nucleic acid in an effective amount for preventing the asthma or allergy. In related embodiments of this method, an asthma/allergy medicament is also administered to the subject. In these latter instances, the amount of the immunostimulatory nucleic acid administered may be that amount necessary to reduce the effective dose of the asthma/allergy medicament which is required to treat or prevent the asthma or allergy.

Thus, in these embodiments, the immunostimulatory nucleic acid potentiates the effect of the asthma/allergy medicament. The ability to potentiate the effect of an asthma/allergy medicament is useful since it allows for a reduction in the administered dose of an asthma/allergy medicament with the same or better therapeutic result. As an example, if the dose of the medicament is lowered, then so too are the side-effects of the medicament such as, for example, drowsiness, nervousness, dizziness or, in some instances, sleeplessness. Similarly, the administration of a lowered dose of the asthma/allergy medicament may make the medicament more compatible with the administration of other medicaments such as those which are currently not simultaneously prescribed or administered with asthma or allergy medicaments. In some instances, these include certain medicaments which are prescribed for depression, psychiatric or emotional conditions or Parkinson's disease and which contain monoamine oxidase inhibitor (MAOI). Similarly, the ability to potentiate the effect of the asthma/allergy medicament, thereby leading to a decreased effective dose, is useful for

treating a wide range of subjects who have previously been contraindicated for such treatment, including subjects with heart disease or diabetes, subjects who have difficulty in urinating due to prostate gland enlargement, and subjects who are pregnant or who are nursing (i.e., breast-feeding). Thus, the invention provides a method for administering to a subject a  
5 dose of an asthma/allergy medicament which if administered alone, or if administered without previous administration of an immunostimulatory nucleic acid to the same subject, would be ineffective (and would be considered sub-therapeutic).

Subject doses of the compounds described herein typically range from about 0.1  $\mu$ g to 10,000 mg, more typically from about 1  $\mu$ g/day to 8000 mg, and most typically from about 10  
10  $\mu$ g to 100  $\mu$ g. Stated in terms of subject body weight, typical dosages range from about 0.1  $\mu$ g to 20 mg/kg/day, more typically from about 1 to 10 mg/kg/day, and most typically from about 1 to 5 mg/kg/day.

In some instances, a sub-therapeutic dosage of the immunostimulatory nucleic acid and the asthma/allergy medicament are used. It has been discovered according to the  
15 invention, that when the two classes of drugs are used together, they can be administered in sub-therapeutic doses and still produce a desirable therapeutic result, a "sub-therapeutic dose" as used herein refers to a dosage which is less than that dosage which would produce a therapeutic result in the subject, if administered alone. Thus, the sub-therapeutic dose of an asthma/allergy medicament is one which would not produce the desired therapeutic result in  
20 the subject. Therapeutic doses of asthma/allergy medicaments are well known in the field of medicine for the treatment of asthma and allergy. These dosages have been extensively described in references such as Remington's Pharmaceutical Sciences, 18th ed., 1990; as well as many other medical references relied upon by the medical profession as guidance for the treatment of asthma and allergy. Therapeutic dosages of immunostimulatory nucleic acids,  
25 have also been described in the art and methods for identifying therapeutic dosages in subjects are described in more detail above.

In other aspects, the method of the invention involves administering a high dose of an asthma/allergy medicament to a subject, without inducing side effects. Ordinarily, when an asthma/allergy medicament is administered in a high dose, a variety of side effects can occur.  
30 (Discussed in more detail above, as well as in the medical literature). As a result of these side effects, the asthma/allergy medicament is not administered in such high doses, no matter what therapeutic benefits are derived. It was discovered, according to the invention, that such high doses of asthma/allergy medicaments which ordinarily induce side effects can be administered

without inducing the side effects as long as the subject also receives an immunostimulatory nucleic acid. The type and extent of the side effects ordinarily induced by the asthma/allergy medicament will depend on the particular asthma/allergy medicament used.

In other embodiments of the invention, the immunostimulatory nucleic acid is administered on a routine schedule. The asthma/allergy medicament may also be administered on a routine schedule, but alternatively, may be administered as symptoms arise. A "routine schedule" as used herein, refers to a predetermined designated period of time. The routine schedule may encompass periods of time which are identical or which differ in length, as long as the schedule is predetermined. For instance, the routine schedule may involve administration of the immunostimulatory nucleic acid on a daily basis, every two days, every three days, every four days, every five days, every six days, a weekly basis, a bi-weekly basis, a monthly basis, a bi-monthly basis or any set number of days or weeks there-between, every two months, three months, four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, twelve months, etc. Alternatively, the predetermined routine schedule may involve administration of the immunostimulatory nucleic acid on a daily basis for the first week, followed by a monthly basis for several months, and then every three months after that. Any particular combination would be covered by the routine schedule as long as it is determined ahead of time that the appropriate schedule involves administration on a certain day.

In some aspects of the invention, the immunostimulatory nucleic acid is administered to the subject in anticipation of an asthmatic or allergic event in order to prevent an asthmatic or allergic event. The asthmatic or allergic event may be, but need not be limited to, an asthma attack, seasonal allergic rhinitis (e.g., hay-fever, pollen, ragweed hypersensitivity) or perennial allergic rhinitis (e.g., hypersensitivity to allergens such as those described herein).

In some instances, the immunostimulatory nucleic acid is administered substantially prior to an asthmatic or an allergic event. As used herein, "substantially prior" means at least six months, at least five months, at least four months, at least three months, at least two months, at least one month, at least three weeks, at least two weeks, at least one week, at least 5 days, or at least 2 days prior to the asthmatic or allergic event.

Similarly, the asthma/allergy medicament may be administered immediately prior to the asthmatic or allergic event (e.g., within 48 hours, within 24 hours, within 12 hours, within 6 hours, within 4 hours, within 3 hours, within 2 hours, within 1 hour, within 30 minutes or within 10 minutes of an asthmatic or allergic event), substantially simultaneously with the



asthmatic or allergic event (e.g., during the time the subject is in contact with the allergen or is experiencing the asthma or allergy symptoms) or following the asthmatic or allergic event.

In some embodiments, the immunostimulatory nucleic acid and the asthma/allergy medicament are both administered to a subject. The timing of administration of both may vary. In some embodiments, it is preferred that the asthma/allergy medicament be administered subsequent to the administration of the immunostimulatory nucleic acid. In some embodiments, the immunostimulatory nucleic acid is administered to the subject prior to as well as either substantially simultaneously with or following the administration of the asthma/allergy medicament. The administration of the immunostimulatory nucleic acid and the asthma/allergy medicament may also be mutually exclusive of each other so that at any given time during the treatment period, only one of these agents is active in the subject. Alternatively, and preferably in some instances, the administration of the two agents overlaps such that both agents are active in the subject at the same time.

In some embodiments, the immunostimulatory nucleic acid is administered on a weekly or biweekly basis and the asthma/allergy medicament is administered more frequently (e.g., on a daily basis). However, if the dose of immunostimulatory nucleic acid is reduced sufficiently, it is possible that the immunostimulatory nucleic acid is administered as frequently as the asthma/allergy medicament, albeit at a reduced dose.

In other aspects, the invention relates to kits that are useful in the treatment of asthma and/or allergy. One kit of the invention includes a sustained release vehicle containing an immunostimulatory nucleic acid and a container housing an asthma/allergy medicament and instructions for timing of administration of the immunostimulatory nucleic acid in the asthma/allergy medicament. A sustained release vehicle is used herein in accordance with its prior art meaning of any device which slowly releases the immunostimulatory nucleic acid.

Such systems can avoid repeated administrations of the compounds, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Patent 5,075,109. Delivery systems also include non-polymer systems that are: lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono-di- and tri-glycerides; hydrogel release systems; sylastic systems; peptide based systems; wax coatings;

compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which an agent of the invention is contained in a form within a matrix such as those described in U.S. Patent Nos. 4,452,775, 4,675,189, and 5,736,152, and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Patent Nos. 3,854,480, 5,133,974 and 5,407,686. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

The asthma/allergy medicament is housed in at least one container. The container may be a single container housing all of the asthma/allergy medicament together or it may be multiple containers or chambers housing individual dosages of the asthma/allergy medicament, such as a blister pack. The kit also has instructions for timing of administration of the asthma/allergy medicament. The instructions would direct the subject having asthma/allergy or at risk of asthma/allergy to take the asthma/allergy medicament at the appropriate time. For instance, the appropriate time for delivery of the medicament may be as the symptoms occur. Alternatively, the appropriate time for administration of the medicament may be on a routine schedule such as monthly or yearly.

Another kit of the invention includes at least one container housing an immunostimulatory nucleic acid and at least one container housing an asthma/allergy medicament and instructions for administering the compositions in effective amounts for inducing a synergistic immune response in the subject. The immunostimulatory nucleic acid and asthma/allergy medicament may be housed in single containers or in separate compartments or containers, such as single dose compartments. The instructions in the kit direct the subject to take the immunostimulatory nucleic acid and the asthma/allergy medicament in amounts which will produce a synergistic immune response. The drugs may be administered simultaneously or separately as long as they are administered close enough in time to produce a synergistic response.

In other aspects of the invention, a composition is provided. The composition includes an immunostimulatory nucleic acid and an asthma/allergy medicament formulated in a pharmaceutically-acceptable carrier and present in the composition in an effective amount for preventing or treating an immune or inflammatory response associated with exposure to a mediator of asthma or allergy. The effective amount for preventing or treating an immune or inflammatory response is that amount which prevents, inhibits completely or partially the induction of the immune or inflammatory response or prevents an increase in the immune or

inflammatory response associated with asthma or allergy. An immune or inflammatory response associated with asthma or allergy includes an induction in IgE, an increase in Th2 cytokines, etc. A mediator of asthma or allergy includes asthma initiators and allergens. An example of a composition is one which comprises an immunostimulatory nucleic acid, such as a CpG nucleic acid, and an asthma/allergy medicament, such as an anti-IgE agent (e.g., an anti-IgE antibody or antibody fragment). Such a composition can be administered to a subject on a routine basis such as monthly, bimonthly, or quarterly.

For any compound described herein a therapeutically effective amount can be initially determined from cell culture assays. For instance the effective amount of immunostimulatory nucleic acid useful for inducing B cell activation can be assessed using the in vitro assays with respect to stimulation index in comparison to known immunostimulatory acids. The stimulation index can be used to determine an effective amount of the particular oligonucleotide for the particular subject, and the dosage can be adjusted upwards or downwards to achieve the desired levels in the subject. Therapeutically effective amounts can also be determined from animal models. A therapeutically effective dose can also be determined from human data for immunostimulatory nucleic acids which have been tested in humans (human clinical trials have been initiated) and for compounds which are known to exhibit similar pharmacological activities, such as other adjuvants, e.g., LT and other antigens for vaccination purposes. The applied dose can be adjusted based on the relative bioavailability and potency of the administered compound. Adjusting the dose to achieve maximal efficacy based on the methods described above and other methods as are well-known in the art is well within the capabilities of the ordinarily skilled artisan. Most of the asthma/allergy medicaments have been identified. These amounts can be adjusted when they are combined with immuno-stimulatory nucleic acids by routine experimentation.

The formulations of the invention are administered in pharmaceutically acceptable solutions, which may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, adjuvants, and optionally other therapeutic ingredients.

Asthma/allergy medicaments and immunostimulatory nucleic acids can be administered by any ordinary route for administering medications. Preferably, they are inhaled, ingested or administered by local routes (such as nasal drops) or by systemic routes. Systemic routes include oral and parenteral. Inhaled medications are preferred in some embodiments because of the direct delivery to the lung, the site of inflammation, primarily in

asthmatic patients. Several types of metered dose inhalers are regularly used for administration by inhalation. These types of devices include metered dose inhalers (MDI), breath-actuated MDI, dry powder inhaler (DPI), spacer/holding chambers in combination with MDI, and nebulizers. As used herein, delivery to the nasal passages or the lungs via nasal drops or inhalation are referred to as local administration. Although it is possible that delivery to the lung (e.g., via inhalation) can eventually result in systemic delivery of the agent, the administration is still considered "local" in the sense that the majority of the agent is initially presented to the lung tissue or the nasal passages, prior to any secondary systemic effects. In some preferred embodiments, the immunostimulatory nucleic acid is administered locally, such as for example by nasal drops or inhalation.

For use in therapy, an effective amount of the immunostimulatory nucleic acid can be administered to a subject by any mode that delivers the nucleic acid to the desired surface, e.g., mucosal, systemic. "Administering" the pharmaceutical composition of the present invention may be accomplished by any means known to the skilled artisan. Preferred routes of administration include but are not limited to oral, parenteral, intramuscular, intranasal, intratracheal, inhalation, ocular, vaginal, and rectal.

For oral administration, the compounds (i.e., immunostimulatory nucleic acids, asthma/allergy medicament, other therapeutic agent) can be formulated readily by combining the active compound(s) with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject to be treated. Pharmaceutical preparations for oral use can be obtained as solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Optionally the oral formulations may also be formulated in saline or buffers for neutralizing internal acid conditions or may be administered without any carriers.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. Microspheres formulated for oral administration may also be used. Such microspheres have been well defined in the art. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.* gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch. Techniques for preparing aerosol delivery systems are well known to those of skill in the art. Generally, such systems should utilize components which will not significantly impair the biological properties of the therapeutic, such as the immunostimulatory capacity of the nucleic acids (see, for example, Sciarra and Cutie, "Aerosols," in Remington's Pharmaceutical Sciences, 18th edition, 1990, pp 1694-1712; incorporated by reference). Those of skill in the art can readily determine the various parameters and conditions for producing aerosols without resort to undue experimentation.

The compounds, when it is desirable to deliver them systemically, may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active compounds may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal or vaginal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

Suitable liquid or solid pharmaceutical preparation forms are, for example, aqueous or saline solutions for inhalation, microencapsulated, encochleated, coated onto microscopic gold particles, contained in liposomes, nebulized, aerosols, pellets for implantation into the skin, or dried onto a sharp object to be scratched into the skin. The pharmaceutical

compositions also include granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, drops or preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above. The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. For a brief review of methods for drug delivery, see Langer, *Science* 249:1527-1533, 1990, which is incorporated herein by reference.

The immunostimulatory nucleic acids and asthma/allergy medicament may be administered per se (neat) or in the form of a pharmaceutically acceptable salt. When used in medicine the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically acceptable salts thereof. Such salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene sulphonic, tartaric, citric, methane sulphonic, formic, malonic, succinic, naphthalene-2-sulphonic, and benzene sulphonic. Also, such salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group.

Suitable buffering agents include: acetic acid and a salt (1-2% w/v); citric acid and a salt (1-3% w/v); boric acid and a salt (0.5-2.5% w/v); and phosphoric acid and a salt (0.8-2% w/v). Suitable preservatives include benzalkonium chloride (0.003-0.03% w/v); chlorobutanol (0.3-0.9% w/v); parabens (0.01-0.25% w/v) and thimerosal (0.004-0.02% w/v).

The pharmaceutical compositions of the invention contain an effective amount of an immunostimulatory nucleic acid and optionally asthma/allergy medicament and/or other therapeutic agents optionally included in a pharmaceutically-acceptable carrier. The term “pharmaceutically-acceptable carrier” means one or more compatible solid or liquid filler, dilutants or encapsulating substances which are suitable for administration to a human or other vertebrate animal. The term “carrier” denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being commingled with the compounds of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficiency.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by

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We claim:

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